

# The Botanical Review

Interpreting Botanical Progress

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Volume I  
1935

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Published Monthly at  
Lime and Green Streets, Lancaster, Pa.





# The Botanical Review

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# THE BOTANICAL REVIEW

VOL. I

JANUARY, 1935

No. 1

## POSSIBILITIES IN PLANT VIRUS CLASSIFICATION

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In respect to classification, the virus diseases of plants stand in sharp contrast to diseases caused by fungi. The latter are grouped in elaborate classifications based for the most part on the natural relationships of their etiological agents. Such a classification of virus diseases is not feasible at the present time, because little is known of the agents causing these diseases. A satisfactory classification of viruses may not be possible until it is proved whether they represent a group of minute living organisms or a series of non-living substances, and a more suitable technique than is now available for their study has been devised. While our present knowledge is too meager to permit the arrangement of plant viruses into groups on a well-founded basis of natural relationships, recent studies justify the hope that a fairly useful classification may soon be possible. Several methods of identifying and grouping viruses have been suggested, but all are somewhat limited in applicability. This paper will attempt to discuss these methods and the bearing of recent work on problems of classification.

### SOME METHODS OF DIFFERENTIATING AND CLASSIFYING VIRUSES

Johnson (27, 28) has recommended a method employing physical and chemical properties, such as thermal death point, retention of activity *in vitro*, and reaction to chemicals, along with symptom manifestations in different host plants. Quanjer (56) has emphasized the value of histological and cytological symptoms for the identification and classification of potato viruses. Elze (19) and Storey (76) have suggested a classification of viruses by means of their insect relationships. Elze separates all viruses into two groups on the basis of whether or not they are transmitted by

insects. Those transmitted by insects are further separated on the basis of whether they are transmitted by one, or more than one, insect. The viruses transmitted by one insect are still further separated into groups having long and short incubation periods in their vectors. Such a classification seems of doubtful value, since it is generally believed that most, if not all, plant viruses are transmitted by insects. Those not now known to be so transmitted do not form a natural group. A division on the basis of transmission by one or by more than one insect also seems to separate viruses that are closely related. Most viruses that are spread by several insects are infectious enough to be transmitted mechanically. Some, however, such as that of potato leaf roll (19, 65, 87, 88), Fiji disease of sugar-cane (49, 50), and curly top of sugar beets (2, 20), seem to enter into a specific relationship with more than one insect. Far too little is known regarding incubation periods of viruses in insects to permit a classification on this basis at the present time.

Storey (76) suggests that in manner of transmission may be found an important criterion for the differentiation of viruses. He regards a specific insect vector of a virus as no less infected than the plant host and notes a remarkably close agreement between a grouping of viruses on the basis of symptom manifestation and a grouping according to the type of insect vector. Storey points out that mosaics are generally transmitted by aphids and yellows diseases by jassids. However, there are some mosaic diseases, such as the yellow bean mosaic of Haiti transmitted by *Empoasca fabalis* De Long (69), and the rice mosaic of Japan transmitted by *Nephotettix apicalis* Motsch (83), that are spread by jassids, and some diseases apparently of the yellows type, such as leaf roll of potato transmitted by *Myzus persicae* Sulz. (65), and the yellow-flat disease of lilies transmitted by *Aphis gossypii* Glover (23), that are spread by aphids. In spite of certain limitations of the method, Storey is undoubtedly correct in his views on the importance of insect relationships as useful criteria for the differentiation and classification of viruses.

#### IMPORTANCE OF BIOLOGICAL CARRIERS

The mechanical spread of viruses by insects is of little or no significance from the standpoint of natural relationships. A

highly infectious virus is likely to be spread by any insect that feeds on infected plants. Some fifty different species, including both aphids and jassids, are reported to spread the mosaic of onions (17) which is readily transmitted mechanically. This knowledge is of little value from the standpoint of virus classification. It is information regarding biological relationships that is useful for this purpose. Whether such relationships consist of infection of insects by the viruses they transmit, as is believed by Storey (76), or in an association of some other nature need not be discussed here. That an intimate specific relationship does exist is generally agreed. Evidence supporting this view is found in studies on insect spread of viruses not mechanically transmissible, on long retention of viruses by certain vectors, on incubation periods of viruses in vectors, and on other insect relationships.

A considerable number of virus diseases not known to be sap-transmissible are spread by insects. Aster yellows, carried by the leafhopper *Cicadula sexnotata* Fallen (35), is a good example of such a disease. In spite of many efforts to transfer the disease by rubbing or injecting sap from diseased plants into healthy plants, it has never been transmitted mechanically except by grafting. The vector, however, transmits the disease very readily. This malady also furnishes a good example of the retention of a virus by a vector. Newly hatched nymphs of *Cicadula sexnotata*, if allowed to become infective by feeding on yellowed aster plants for a few hours, usually retain the yellows virus throughout life even when cultured on rye or other plants immune to yellows. In a few instances it has been proved that viruses are transmitted by vectors only after an incubation period. The insects can not infect healthy plants immediately after first feeding on diseased plants. A definite period of time must elapse between the hour in which they first pick up virus and the hour in which they are first capable of transmitting it. The virus of curly top of sugar beets requires a minimum incubation period of from 4 to 48 hours in the leafhopper *Eutettix tenellus* Baker (63, 70), depending on the temperature at which the insect is held. The period is shortened by high temperatures. The viruses of potato leaf roll (18, 64, 65), streak disease of corn (73), bunchy top of abaca (51), and mosaic of peas (53) have incubation periods of similar lengths in their insect vectors. Longer incubation periods have

been reported for the virus of aster yellows in *Cicadula sexnotata* (34, 35), the virus of yellow spot of pineapple in *Thrips tabaci* Lind. (43, 44), and the virus of spotted wilt of tomato in *Frankliniella insularis* Frankl. (1) and *Thrips tabaci* (67). The aster-yellows and yellow-spot viruses require periods of about 10 days, while the spotted-wilt virus requires from 5 to 7 days. The incubation periods suggest that these viruses may pass through some stage of development, or at least may multiply, in their vectors.

Equally good evidence of a biological relationship is brought by studies on the relation between age of vectors and their ability to become infective. Adults of the species *Frankliniella insularis* (61) are unable to transmit the virus of spotted wilt unless they are hatched from infected pupae. Both larvae and adults can transmit the virus, but only the larvae can pick it up. A similar relationship exists between this virus and its other vector, *Thrips tabaci* (67), and between the yellow-spot virus of pineapples, which may or may not be identical with spotted-wilt virus, and *Thrips tabaci* (43). A somewhat similar relationship is suggested by Rankin's (58) report that very young instars of the first, second, and later generations of *Amphorophora rubi* Kalt. taken from mosaic-diseased raspberry leaves were infective, while their mothers and more mature sisters taken from the same leaves were noninfective. Fukushi's (21) discovery that rice-mosaic virus is transmitted through the eggs of its leafhopper vector, and Storey's (78) discovery that in the leafhopper species *Cicadulina mbila* Naude ability to transmit streak of corn is inherited as a simple dominant sex-linked Mendelian factor, and that individuals lacking this factor may become infective by needle inoculations (80) although they do not become infective by feeding on diseased plants, bring further evidence of the delicate biological relationships that exist between insects and viruses.

It is possible, and even probable, that the highly infectious viruses, as well as those not readily sap-transmissible, are biologically carried by one or more insects. Evidence of this is, however, difficult to obtain because in such cases there is no easy way of separating the mechanical carriers from the biological carriers. Viruses that are not mechanically transmitted, or at least are not easily so transmitted, are spread by biological carriers only. In

such instances the biological relationship is not obscured by mechanical transmissions. Up to the present time no highly infectious virus has been shown to be biologically transmitted and proof of such transmission has been given for less than one fourth of the known plant viruses. When biological carrier relationships are better known and have been established for a large number of virus diseases, they may furnish a good basis for differentiation and classification.

Some examples of virus diseases believed to be biologically carried, together with their vectors, are listed below:

Curly top of sugar beets in the United States by *Eutettix tenellus* Baker (70).

Curly top of sugar beets in Argentina by *Agallia sticticollis* Stål. (20).

Mosaic of rice in Japan by *Nephotettix apicalis* Motsch (83).

Yellows of the China aster in the United States by *Cicadula sexnotata* Fallen (35).

Streak of corn in South Africa by *Cicadulina mbila* Naude (72).

Streak of corn in Tanganyika by *Cicadulina zeeae* China (76).

Yellow dwarf of potato in the United States by *Agallia sanguinolenta* Prov. (7).

Fiji disease of sugar cane in Queensland, Australia, by *Perkinsiella saccharicida* Kirk. (49).

Fiji disease of sugar cane in the Philippine Islands by *Perkinsiella vastatrix* Bred. (50).

Yellow bean mosaic in Haiti by *Empoasca fabalis* De Long (69).

False blossom of cranberries in the United States by *Euscelis striatulus* Fall. (15, 16).

Peach yellows in the United States by *Macropsis trimaculata* Fitch (38).

Corn mosaic in Hawaii by *Peregrinus maidis* Ashm. (33).

Potato leaf roll in many different countries by *Myzus persicae* Sulz. (62) and by *Myzus circumflex* Buckton (87).

Sugar cane mosaic in many countries by *Aphis maidis* Fitch (10).

Red raspberry mosaic in the United States by *Amphorophora rubi* Kalt. (58) and *Aphis rubiphila* Patch (57).

Mosaic of peas and other leguminous plants in the United States by *Macrosiphum pisi* Kalt. and *Macrosiphum gei* Koch (53).

Peanut rosette in Africa by *Aphis leguminosae* Theobald (74).

Lily mosaic in the United States and other countries by *Aphis gossypii* Glover (23).

Yellow flat disease of lilies in Bermuda by *Aphis gossypii* Glover (52).

Banana bunchy top disease in Australia by *Pentalonia nigronervosa* Coq. (45).

Bunchy top of abaca in the Philippine Islands by *Pentalonia nigronervosa* Coq. (51).

Spotted wilt of tomato in Australia by *Frankliniella insularis* Frankl. (60).

Spotted wilt of tomato in England by *Thrips tabaci* Lind. (66).

Pineapple yellow spot in Hawaii by *Thrips tabaci* Lind. (42).

Leaf curl of cotton in the Sudan by *Bemisia gossypiperda* Misra and Lamba (31).

Leaf curl of tobacco in Rhodesia and elsewhere by *Bemisia gossypiperda* Misra and Lamba (77, 79).

The above list of biological carriers includes 13 jassids, 10 aphids, 2 thrips, and 1 whitefly. Most of the diseases in this list are known to be transmitted by one insect only and are undoubtedly caused by different virus entities. Several of the diseases are, however, transmitted by more than one insect, as was mentioned previously. The curly top of sugar beets in the United States is spread by the leafhopper *Eutettix tenellus* (2) only, but the curly top of sugar beets in Argentina, where *Eutettix tenellus* does not occur, is transmitted by another leafhopper, *Agallia sticticollis* (20). Whether or not the disease in Argentina is due to the same virus that causes curly top in the United States is not known. It seems identical from the standpoint of symptomatology. The Fiji disease of sugar cane is transmitted by *Perkinsiella saccharicida* in Queensland, Australia, (49), and by *P. vastatrix* in the Philippine Islands (50). Since the symptoms of this disease are distinct from those of all other known plant diseases, there can be little doubt that the virus in Australia is identical, or very closely related, to that prevalent in the Philippine Islands. If they are identical, we have here an excellent example of a virus that it biologically transmitted by two different, though closely related, insect species. A similar case is reported by Storey (76) for the streak disease of corn which is transmitted by both *Cicadulina mbila* and *C. zeae* in Tanganyika where the two species exist side by side. Storey proved that the virus in a plant infected by means of *C. mbila* may be taken up and transmitted by *C. zeae*. Osborn (53) has shown that pea mosaic, although not transmitted by *Aphis rumicis* L., is carried by both *Macrosiphum pisi* and *M. gei*. The spotted wilt of tomato, transmitted by *Frankliniella insularis* (60) and *Thrips tabaci* (66), is another good example of a virus that is biologically carried by two different insects. The leaf-roll disease of potato is reported to be spread by the following aphids:



*Myzus persicae*, *Aphis rhamni* Boyer (19), *Macrosiphum pelargonii* Kalt. (88), *M. gei* (87), *Myzus pseudosolani* Theobald (65), and *M. circumflexus* (87). Since the virus of this disease is not mechanically transmissible, it is assumed that a biological relationship exists between it and each of these aphid vectors. There are, on the other hand, a few instances of the biological transmission of two very different viruses by the same insect. *Aphis gossypii* transmits both the mosaic (23) and the yellow-flat (52) diseases of lily. These diseases are believed to be distinct. *Myzus persicae* is a vector of dahlia mosaic (14) and potato leaf roll (62), two very distinct diseases. *Pentalonia nigronervosa* transmits bunchy top of abaca in the Philippine Islands (51) and bunchy top of banana in Australia (45). These two diseases are very much alike, but the Philippine virus is not transmissible to bananas, either in the field or experimentally, by the vector which readily transmits the bunchy top virus to bananas in Australia. A similar instance is reported for *Cicadula sexnotata* which transmits both the eastern and the western types of aster yellows (37) in the United States. The two diseases are indistinguishable on asters, but the western or California type of yellows is readily transmitted to celery and Zinnia while the eastern type of yellows does not go to these plants.

It is evident from the above discussion that some of the viruses which are known to be biologically carried are not readily differentiated on the basis of vector relationships alone. Although such relationships are highly important, they are usually not sufficient for purposes of classification unless considered in connection with other characteristics of the viruses and the diseases they produce. This examination of some of the methods recommended for the classification of plant viruses leads to the conclusion that, while no one method is now applicable to all viruses, each method can be used to advantage with certain of them. As has already been stated by Quanjer (56) and Smith (68), much more information must be collected before a satisfactory and stable classification will be possible. It is, however, not to be expected that efforts in this direction will await completion of all the studies that may be necessary for a final classification. The arrangement of related viruses into groups is being accomplished gradually as new information

becomes available and new methods for virus differentiation are devised.

#### EVIDENCE OF RELATIONSHIPS AMONG THE PLANT VIRUSES

When a virus is found that affects some plant not previously known to be attacked by other viruses, or that produces in plants susceptible to other viruses symptoms distinguishable from those characteristic of diseases caused by known viruses, it is usually described as new and the disease it produces as a new disease without any attempt to relate it to other members of the virus group. This practice has led to the recognition and naming of many diseases caused by closely related viruses. Recent studies indicate that there are prevalent in nature a considerable number of viruses that are only slightly different from other viruses and may therefore be considered strains of the latter. In the opinion of the writer, these studies covering a wide range of diseases fully justify the conception of strains among the plant viruses.

Mention has already been made of the fact that a disease known as bunchy top affects bananas in Australia, and that a malady with similar symptoms affects abacas but not bananas in the Philippine Island, and of the eastern and western types of aster yellows in the United States that are identical as far as the vector and symptoms on aster plants are concerned but have slightly different host ranges. Two leaf-curl diseases (5) affecting red and black raspberries, and designated by Bennett as alpha curl and beta curl, are transmitted by *Aphis rubiphila* and are similar symptomatically except on the purple variety Columbian. One of them does not, however, attack the variety Black Cumberland. Storey (76) has demonstrated the existence of two strains of sugar cane mosaic that differ in host range. Both are transmitted by *Aphis maidis*.

Summers (82) reports the presence of four types of sugar cane mosaic in Louisiana. They are distinguished by symptom differences on different varieties of cane. Storey and McClean (75) found several different types of streak in East Africa. The virus of each disease shows a specialization for its own host. Thus the virus from corn streak produces a severe and permanent disease in corn, but only a transitory infection in Uba cane. The Uba cane streak virus produces a mild but permanent disease in cane. In corn it causes a very mild disease that tends to become sup-

pressed with the growth of the plant. The wild grass, *Digitaria horizontalis* Willd., is affected by a streak virus that differs from both the corn and the cane viruses. All of these viruses are spread by the same vector, *Cicadulina mbila*. A disease very similar to Storey's streak of corn is prevalent in the Hawaiian Islands. It was shown to be distinct from sugar cane mosaic (36) and to be transmitted from corn to corn, but not from corn to cane, by *Peregrinus maidis* (33). Stahl (71), working in Cuba, described a similar disease of corn which is also transmitted by *Peregrinus maidis*. *Cicadulina mbila* does not occur in Hawaii or Cuba, but *Peregrinus maidis* does occur in East Africa. It does not, however, transmit the East African streak of corn. These corn diseases of Hawaii, Cuba, and Africa are so similar in symptom manifestations that it is difficult to believe they are not closely related. The Hawaiian disease is readily diagnosed by the presence of characteristic inclusion bodies in affected cells of both stems and leaves (32). Since neither Stahl nor Storey has studied the stripe or streak diseases cytologically, nothing is known regarding them from the standpoint of cellular pathology. Conspicuous inclusion bodies should be found in cells of plants infected with stripe or streak if these diseases are related to the Hawaiian disease of corn. As was stated above, the Fiji disease of sugar cane in Australia is spread by the leafhopper *Perkinsiella saccharicida* (49). In the Philippine Islands where *P. saccharicida* does not occur, a similar, if not identical, disease of sugar cane is spread by *P. vastatrix* (50). Two types of kroepoek disease of tobacco are described by Kerling (29) and three types by Thung (85). All are transmitted by the same whitefly and are much alike except for severity of symptoms.

Carsner and Lackey have described attenuated strains of the sugar beet curly-top virus (11, 12, 40, 41). The strains were obtained by passage of ordinary curly-top virus through resistant varieties of sugar beets or through resistant weed species. Attenuated strains of tobacco mosaic obtained from infected plants or tissues after incubation at high temperatures have also been described (24, 26).

Böhme (8) reports the isolation of four types of X- (latent virus of potato) and three types of Y-virus (veinbanding mosaic

of potato) from potato. The types in both the X- and the Y-groups produce slightly different symptoms in certain host plants. One of the X-virus strains could not be mechanically transmitted to certain potato varieties except by grafting, while two of the others were transmissible by rubbing. One of the Y-virus strains was found to have a thermal death point of about 50° C., whereas another Y-virus strain was still infectious when heated to 55° C. The virus of yellow mosaic of tomato was found by Stover and Vermillion (81) to be inactivated at about 83° C., while that of ordinary tobacco mosaic was inactivated at about 90° C. The yellow mosaic is presumed to have been a strain of tobacco mosaic, since it caused streak on tomato when in combination with the latent virus of potato. Two strains of Abutilon mosaic differing in severity of symptoms are reported by Keur (30). Venkata Roa and Gopala Iyengar (86) have recently described two symptomatically distinct types of the spike disease of sandal.

That the different virus strains in the groups listed above may be related in the sense of one strain being derived from another is suggested by the fact that certain strains differ only in symptom intensity, in host range, in insect vector, in thermal death point, or in infectivity, but even better evidence of genetic relationships is now available.

McKinney (46) was the first to show that a yellow strain of tobacco mosaic may be obtained from the bright yellow spots that occasionally occur in the leaves of tobacco plants infected by the ordinary green tobacco mosaic. He suggested that the yellow strain may arise as a mutation from the green strain (47). McKinney (48) has likewise shown that a yellow strain of wheat mosaic may be isolated from wheat plants infected with the ordinary green-mosaic virus of wheat. Jensen (25) found that a whole series of yellow and necrotic strains of tobacco mosaic may be obtained from the bright yellow spots, and that the spots occur in the leaves of plants infected by ordinary tobacco-mosaic virus that has apparently been freed of any contaminating viruses by successive passage through primary necrotic lesions in leaves of *Nicotiana glutinosa* L. Some of the yellow strains isolated by Jensen appear to be identical with yellow strains of tobacco mosaic

occurring in nature. Others differ from any of the yellow strains that have been described previously. One of Jensen's strains causes symptoms similar to aucuba mosaic of tomato on tomato and tobacco plants and, like the latter, produces necrotic primary lesions on mature leaves of *N. sylvestris* Spegaz. and Comes and certain other host plants. Several of Jensen's strains are much less infectious than the virus of green tobacco mosaic from which they were derived.

Price (54) has isolated several different strains of yellow cucumber mosaic from bright yellow spots that occasionally occur in leaves of tobacco plants infected with ordinary cucumber-mosaic virus. All of these strains, as well as the ordinary green cucumber-mosaic virus, produce necrotic primary lesions in the leaves of cowpea plants of the variety Black Eye. None of them cause mottling in the cowpea. Among the necrotic lesions regularly produced on cowpea leaves by the green-mosaic virus, Price found two chlorotic lesions. From these he isolated a cucumber-mosaic virus that is systemic and causes mottling in cowpeas. He thus obtained experimentally a strain of cucumber-mosaic virus that differs from all other known strains in respect to symptoms on cowpeas. This strain differs symptomatically from the green cucumber-mosaic virus from which it was derived to about the same extent that the streak virus of corn in East Africa differs from Storey's streak virus of cane.

The work of McKinney, Jensen and Price proves that variant strains of both the tobacco- and the cucumber-mosaic viruses may be isolated from plants infected with viruses causing the ordinary types of these diseases, and, in addition, brings evidence that the variants are derived from the ordinary well known types. These results support the view that the variant strains isolated, as well as the several strains of both viruses, known to occur in nature, are genetically related. If this be true, the viruses causing diseases that differ only slightly from other diseases should be grouped together and treated as members of a naturally related family. We would thus assemble a tobacco-mosaic virus group, a cucumber-mosaic virus group, a corn-streak virus group, a sugar cane mosaic virus group, an X-virus (latent virus of potato) group, a Y-virus (potato veinbanding mosaic) group, etc.

## NEW METHODS FOR DIFFERENTIATING PLANT VIRUSES

This brings us to a consideration of some further methods available for testing relationships suspected to exist between viruses causing diseases that resemble each other. Evidence indicating that certain plant viruses, or substances intimately associated with these viruses, are antigenic has been accumulating during recent years (3, 4, 6, 13, 22, 55). For example, there is now good reason to believe that the viruses of tobacco mosaic, cucumber mosaic, and tobacco ring-spot have this capacity. Each is readily distinguished from the other two antigenically. Chester (13) has recently shown that Jensen's tobacco-mosaic virus strains have similar antigenic properties, and that the different cucumber-mosaic virus strains isolated by Price are also alike in this respect. The strains in the tobacco group are, however, entirely distinct antigenically from strains in the cucumber-mosaic group, although certain members of the two groups show remarkably similar symptom manifestations. The results presented by Jensen and Price in support of the view that virus strains isolated from plants having tobacco mosaic or cucumber mosaic are related to tobacco-mosaic virus and cucumber-mosaic virus respectively, are thus confirmed. While the serological method may not prove suitable for the study of all plant viruses, it nevertheless offers interesting possibilities for virus classification.

A second method for testing virus relationships depends on the fact that the infection of a plant by one strain of a virus will, in some cases at least (9, 39, 59, 84), protect the plant against infection by a related strain, while it will give no protection against an unrelated strain. This method has been successfully used for the differentiation of strains of tobacco-mosaic virus from cucumber-mosaic virus and tobacco ring-spot virus (39). It will doubtless prove useful for the testing of relationships in other groups. The two methods are similar in that they depend on immunological reactions; the first on an immunological reaction in an animal, the second on an immunological reaction in a plant. Therefore, they offer the means for a more convenient and direct test of virus relationships than do some of the other criteria. It is believed that by using these two methods, in combination with other methods discussed, it may soon be possible to arrange most, if not all, of the

plant viruses into naturally related groups which may serve as a basis for the classification of virus diseases.

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# THE STRUCTURE OF PROTOPLASM

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The earliest work on protoplasm, done by such pioneers as Rösel von Rosenhof, Dujardin, von Mohl, and Purkinje was on living material. Then followed the work of the cytologists on fixed material. Much of interest and value resulted from these latter studies, notably the discovery of chromosomes and the phenomenon of mitosis. The new century ushered in a "new cytology" and with it not only a return to living material, but a new view-point based on modern physical and chemical interpretations. This change came about for a number of reasons. The now old cytology found itself, at least temporarily, in a *cul de sac*; the chemists were just beginning to offer their first substantial contributions to the study of colloidal systems, in particular, jellies; and the cytologist had come to realize that if he went no further than what he could see in his microscope, he would not get far, any more than would have the physicist had he stopped at what he could see and make a model of. Such methods, which dominated the mechanical interpretations of natural phenomena during the nineteenth century, had inevitably to give way to interpretations based on behavior, whether the behavior of electrons, colloidal systems, or protoplasm. One can not rest content with visible structure.

The new cytology, from a strictly physiological point of view, may be said to have started with the work of Claude Bernard; from the chemical point of view, it began with the work of H. R. Proctor on "The Structure of Organic Jellies" (34).

Protoplasm was viewed by the older workers either as a fine granular suspension or as a framework of fibers, the latter forming a continuous net or an entanglement of discontinuous fibrils.

In 1894 Bütschli advanced his alveolar hypothesis of protoplasmic structure. He introduced the word *alveolus* (a small alveole or cavity) to indicate symmetrically arranged globules resembling small vacuoles and which, because of pressure, have assumed an angular form. Neither alveolus nor vacuole is, however, a well chosen term as there are no cavities or empty sacs in protoplasm. The alveolar structure is, in reality, that of an

emulsion, the globules of which are numerous, compact and under pressure; they, therefore, assume an angular and uniform shape and are symmetrically arranged, as in the case of the "cells" of a honeycomb.

There is no truth to the contention often made that the alveolar structure of protoplasm is always an artifact. It is clearly and perfectly presented in the ectoplasm of certain protozoa, e.g., *Euplotes*, but it is not of universal occurrence, nor of fundamental significance, as Bütschli maintained.

The emulsion hypothesis of protoplasmic structure is accepted by many (8, 9) though unfortunately not, in the author's opinion, wisely so. In its coarser structure, protoplasm, as seen through the microscope, is an emulsion, a dispersion of globules of varying size and distribution in an aqueous medium. When the globules are numerous, compact, angular and symmetrical, alveolar protoplasm results. When the globules are relatively large, uniform and spherical, they are termed *alveolar spheres* (55). When the globules appear to be "cavities" of the nature of small sacks, the structure is termed vacuolar (6). But it is impossible to differentiate clearly between a vacuole, a sack, a globule, an alveolus, or an alveolar sphere. All of these structures are but modifications of one and the same thing, namely, a globule; they all become, therefore, the dispersed phase of an emulsion.

One of the most interesting and significant among recent contributions to our knowledge of the protoplasmic emulsion is an observation by Spek (46, 48) who observed the fusion of several of the most minute of protoplasmic granules to form larger liquid globules with discernible contours. Thus does the "granular" structure fall under the heading of an emulsion. (Some solid granules occur in protoplasm, e.g., crystals). We, therefore, recognize one main type of visible structure in living protoplasm, namely, an emulsion. The various distinctive names which have been given to this structure indicate, in part, the actual particular configuration assumed by the emulsion and, in part, the author's interpretation of it as it exists in the particular material he has investigated.

#### *The emulsion hypothesis.*

There is no doubt that protoplasm, superficially viewed, is an emulsion. Doubt exists only as to the function and fundamental

nature of this emulsion. The emulsion hypothesis got a firm hold in biology for two reasons. First, protoplasm as seen through the microscope is quite evidently an emulsion and the colloidal structure of jellies was once thought to be a fine emulsion. The latter idea gave rise to the misleading word "emulsoid." It was very natural, therefore, for biologists to assume that the ultimate and hidden structure of protoplasm is an emulsion like the coarser and visible one, only finer. Let us first recall what happened to the emulsion hypothesis of the structure of jellies. Ellis (12) found that fine and pure emulsions are "model suspension colloids" and not of the jelly type at all. Hatschek (20), in a search for a possible mechanism in emulsions which would explain such gel properties as elasticity, analyzed the situation mathematically and concluded, "the theory that gels consist of two liquids must be pronounced untenable."

The first substantial contribution to the emulsion hypothesis of protoplasmic structure was that of Clowes (8) who evolved an ingenious theory of protoplasmic permeability. He assumed that the outer layer of protoplasm is an ultramicroscopic emulsion near the reversal point. When the emulsion swings slightly to one side or the other, toward the oil-in-water or water-in-oil state, it becomes more or less permeable to water soluble substances such as salts. The hypothesis nicely explains certain features of the permeability of protoplasm. Particularly convincing is Clowe's discovery that the proportion of sodium and calcium which keeps an emulsion at the reversal point is exactly that which exists in seawater, in blood and other physiological solutions.

A second substantial support to the emulsion hypothesis of protoplasmic structure, in reference to the surface layer, came from Dixon and Clark (10). They found that an electrical stimulus affects emulsions in the same way as it does protoplasm. An electric current will cause an emulsion, originally almost impermeable to ions and water soluble substances, to become fairly permeable, which is the same effect that electric stimuli have on living tissues, namely, they increase permeability. Dixon and Clark conclude, therefore, that an hypothesis which explains two such apparently unconnected and remarkable phenomena of the antagonistic action of ions on permeability, the hypothesis of Clowes, and the permeability changes produced by electric stimuli,

the work of Dixon and Clark, deserves serious consideration. This is true, yet it may simply mean that two rather diverse types of systems, an emulsion and a living jelly, show similar responses to the same environmental changes.

We are forced to discard the emulsion hypothesis of membrane control, in spite of two substantial facts in its support, that of Clowes and that of Dixon just cited, because of the following reasons. There is no direct evidence whatever of a phase reversal of the protoplasmic emulsion. Electrical conductivity measurements reveal that the conductivity of protoplasm is the same *at all viscosity values*. Blood also shows no change in conductivity in spite of a great increase in viscosity as a result of coagulation. This is true also of gelatin when it sets from a solution to a gel, and on this basis McBain (26) denied the possibility of phase reversal when soap jellies are formed. The whole idea of phase reversal has been discarded as a property of gel-forming systems of which protoplasm is one. It is very unlikely that protoplasm could exist as a living substance if fat were the continuous phase; metabolic reactions take place in aqueous media. As the stability of an emulsion increases with decrease in size of the dispersed particles, an ultramicroscopic emulsion will be extremely difficult to reverse, owing to a great increase in the surface tension of the stabilizing membrane. The amount of fat in the dispersed globules of an ultramicroscopic emulsion is probably insufficient to enclose the aqueous medium. There are, further, some very characteristic properties of protoplasm which cannot be explained on the basis of an emulsion structure. Protoplasm is elastic and emulsions, when pure, are not. Protoplasm coagulates and emulsions do not. When milk coagulates, it is a protein, caseinogen, which coagulates and not the emulsion of butter-fat.

The visible protoplasmic emulsion has its own important rôle to play. In addition to its nutritional properties, it presents a multitude of surfaces, and it is at surfaces chemical reactions take place. The microscopic protoplasmic emulsion may be present even when it is not visible with ordinary optical methods. That this is true is indicated by observations made with the Spierer lens (44, 49). This optical system is a Ziess 1/12" f.l., 1.25 n.a. (.8 n.a. with iris closed), 90x, oil-immersion objective, on the lower lens of which a small metallic mirror has been placed which reflects all direct light,



thus permitting illumination of the material from below, as is customary in ordinary microscopic observations, yet giving a dark-field. The principle of the lens is based on the fact that the light scattered by a colloidal particle is most intense in the direction of the illuminating ray, according to the formula of Rayleigh (43). The Spierer objective is thus a complete dark-field system in itself, but it is used to advantage with a cardioid condenser. All structures seen with the Spierer lens in both protoplasm and cellulose have also been seen with cardioid and light-field optical systems, but less distinctly so. This statement is made because, as dark-field pictures owe their existence to diffraction phenomena, i.e., to the scattering of light, as do X-ray spectrograms, it is natural that question should arise in regard to the reality of structures revealed by dark-field, whether with the cardioid condenser or the Spierer objective. The first fact of significance is that no optical system can reveal structure of any type in a structureless space. The mere presence of diffraction phenomena is evidence of structure. While the structure revealed by diffraction phenomena may not be an exact counterpart of the actual structure, yet a linear orientation of parts in the picture must correspond to linear units in the object, while points indicate either a granular structure or a segmented linear one. A diffraction grating of 10,000 lines per inch viewed with ordinary light field and a 1/2" oil immersion objective, and also viewed with the Spierer lens, shows the same number of lines per linear unit in both cases. There is no duplication by the Spierer lens. In the same manner, the Spierer lens changes nothing in the structure of protoplasm; it merely brings to light what is poorly seen by other methods. Obviously, diffraction lines, halos and like optical effects occur in all optical systems at times, particularly with dark-field illumination.

When apparently homogenous hyaline protoplasm is viewed through the Spierer lens it often presents the picture of an emulsion in which one, the dispersed, phase is brightly illuminated while the other, dispersion medium, remains dark. When the protoplasm is quiet the two substances present a mottled picture, a mosaic. The plant cell nucleus is of a similar mottled appearance; here the structure is often to be seen with ordinary direct illumination. When the protoplasm is under tension, as when formed into a thread, or when streaming, the emulsion assumes a striated appearance due



to a parallel arrangement of the illuminated and now elongated emulsion globules. Under stress, the globules becomes distorted into rods which are oriented end to end, sometimes so close as to appear to form a continuous thread. This structure, first brought out in detail and with strong contrast by the Spierer lens, had been previously revealed, less distinctly though definitely so, by ordinary light-field methods. A photograph by Scarth (36) shows the same structure in the streaming protoplasm of *Spirogyra*.

"Dispersed phase" and "dispersion medium" would be sufficient to designate the two parts of this emulsion, yet it seemed worth while (44), if for no other reason than to be certain that they will be definitely reckoned with, to give Greek names to the parts of this delicate visible protoplasmic emulsion. The brightly illuminated dispersed phase has been termed *phaneroplasm* (*phaneros* = evident), and the invisible, optically empty background or continuous phase, *cryptoplasm* (*cryptos* = hidden).

A number of other terms have been used which may possibly apply to phaneroplasm and cryptoplasm, though the authors of them seem to have reference to parts more comparable to the old idea of a "spongioplasm" (framework) and an "enchylema" (intervening substance). Examples of these are the terms coined by Strasburger, "kinoplasm" or active plasm and "trophoplasm" or nutritive plasm, terms which have been brought back into use by Lloyd & Scarth (23).

The more closely one approaches the ultimate structure of protoplasm, the less easy is it to differentiate vitally between the relative importance of its constituents, but if we attempt to distinguish between phaneroplasm and cryptoplasm from the view-point of their vital significance, then, discontinuity of the former and active streaming of the latter suggest that cryptoplasm, the continuous phase, is the more fundamental of the two.

#### *The protoplasmic framework.*

There has long persisted in the minds of biologists the thought that there must exist a continuous framework of some sort which is the structural background of protoplasm. Life in a dispersion (solution) of isolated units, no matter how complex the mixture, is inconceivable. Both this theoretical concept and actual observations on fixed and stained material indicated the presence in proto-

plasm of a structure, variously described, but in all instances consisting of a meshwork or entanglement of fibers, forming a three-dimensional net or sponge. The idea of continuity in protoplasmic structure is thoroughly sound and is supported by ample evidence, but much, though by no means all, of the cytological support, based on fixed material, given to it, is faulty. The *fibrillar* hypothesis, advanced by Flemming and others, ascribes to protoplasm the structure of an entanglement of fibrils. Flemming elevated these fibrillae, as did Altmann his granules, above the lowly station of mere structural units and viewed them as the seat of the energies on which life depends. The drawings of Flemming of connective tissue, of Heidenhain of muscle and spinal ganglion cells, and preparations of Strasburger (41) depict a fibrillar structure. Such a structure is characteristic of and visible in certain living tissues. Ettisch (13), with the aid of dark-field illumination, finds the construction of sinew to be that of an aggregation of minute fibers.

The fibers so far referred to are of microscopic dimensions but are built up of finer ultramicroscopic, invisible fibers, probably present in living tissue generally.

Linear structural units may be oriented so as to form an entanglement such as exists in a brush-heap, or they may be arranged in a more orderly manner in the fashion of a three-dimensional net. Earlier controversies often centered on the question whether protoplasmic fibers are discontinuous or whether they anastomose to form a *reticulum*. The meshes of the supposed protoplasmic net were said to be from  $1/2$  to  $2\mu$  in size. Whether the purely anatomical framework or the hyaloplasm, "enchylema," which bathes it, is the real living substance, was judged in the favor of the latter.

The concept of a reticulum as the structural framework of protoplasm has persisted in medicine in the widely recognized *stroma* in the red blood cell. The stroma is presumed to be a delicate web-like net, peripherally located. Microdissection studies fail to reveal any such framework either in the large (nucleated) amphibian erythrocyte or in the human corpuscle (39). While the concept of a continuous framework is a justifiable one and finds ample evidence in other material, the red blood cell is simply a sack.

The reticular, fibrillar, net and sponge-like structures seen in fixed protoplasm may be true fibrous coagula or pictures of an emulsion caught in a coagulum; for example, "chromatin granules" on a "linin thread," a structure which has played a prominent rôle in modern cytological and genetical theories of nuclear behavior, is readily produced by a distorted emulsion of large globules with a minimum of dispersion medium (42). The "granules" would then be the points where several globules approach each other and the "linin thread" would be the connecting strands of the dispersion medium. This is shown by comparing a typical drawing of chromatin material, showing "chromatin granules" on a "linin thread," with one or more of the possible configurations of an emulsion. The symmetrical arrangement of the phases of an emulsion might well pass for the picture of a net or reticulum.

With the older concept of a framework as the structural basis of protoplasm in mind but with the realization that the earlier evidence for it is not always sound, let us turn to modern theories.

*The ultramicroscopic structure of protoplasm.*

The ultramicroscopic structure of protoplasm, like that of non-living matter, is obviously not visible, but theories pertaining to it, as to molecular and atomic structure, are based on sound though indirect evidence. We can best approach our problem by a simple analogy. Of two soap solutions, one of low concentration and low viscosity, and one of high concentration and high viscosity, the former was elastic and the latter not; the former held a small metal particle in suspension, while the latter could not support the same particle. It would seem, therefore, that the elastic yet thin soap solution possessed a structure which would account for its elastic qualities and for its ability to support a metal particle, while the thicker yet inelastic soap lacked such a structure. This supposition was supported by microscopic examination. The elastic soap solution contained long and slender crystals, while the other soap resembled chalk dust. We have in the behavior and structure of these two soaps the basis of all generally accepted hypothesis of the structure of jellies. Elastic colloidal systems are built up of linear crystalline units. Their intermeshing gives elasticity and rigidity to liquids which yet flow freely and smoothly. This is structurally possible if we regard the framework of fibers as not

fixed but labile, capable of readjustment and comparable to a loosely put-together brush-heap. A brush-heap is elastic; a sand pile is inelastic.

Before carrying the story of the fibrous structure of protoplasm over to cellulose, investigations on which have yielded much in regard to gel structure in general, let us see how the intermeshed fibrous structure is associated with the protoplasmic emulsion. Milk illustrates the situation almost perfectly. Viewed through the microscope, milk is an emulsion of butter-fat in an aqueous medium. More than this is not visible. When milk coagulates the emulsion plays only a passive part. It is the casein in milk which coagulates. The fluid whey, an aqueous solution of salts, sugars, etc., separates from the casein coagulum. There are thus in milk three quite distinct systems, intimately associated, namely, an emulsion of fat, a dispersion of fibrous units capable of forming a coagulum, and a solution, of salts, etc., permeating the whole. So it is with protoplasm.

Investigations on the structure of cellulose give the best possible insight into modern interpretations of the mechanism underlying the behavior of colloidal jellies, including protoplasm.

#### *The structure of cellulose.*

The cellulose molecule is now thought to be a chain built of rings of anhydrous glucose ( $C_6H_{10}O_5$ ) (28, 50, 51). This latter group has long been known to be the basic unit of cellulose and all higher carbohydrates, but the number and arrangement of the rings in the larger cellulose molecules were not known. It is now believed that in cellulose each ring is joined to its neighbor by an oxygen bridge, and every alternate ring is the reflected image of the one on each side of it, i.e., it is rotated through  $180^\circ$ . Two such rings constitute an anhydrous molecule of the sugar cellobiose ( $C_{12}H_{22}O_{11}$ ). Some forty or more of these rings, so-called glucose "residues," joined in a continuous chain, form the cellulose molecule. The length of the chain is not fixed. It is capable, stoichiometrically at least, of reaching any length. One can not, therefore, speak of a cellulose molecule in the strict sense if by molecule is meant a unit of fixed weight and constitution. A length of forty glucose residues, or twenty times the length of the cellobiose molecule (10.3 A. U.), represents a chain length of about 200 A. U. This is a

minimum. Two or three times this probably more closely represents an average. The "macro-molecule" of the cotton fiber appears to be the longest, 1000 A. U. Physically, the molecules must be regarded as comparatively stiff threads.

The linear cellulose molecule has at each end an apparently unsatisfied valence bond. There is little likelihood that such a free carbon bond actually exists; it rather indicates where our knowledge ends. The bond is possibly satisfied by a univalent (OH) group or joined to an adjoining chain.

The molecular weight of this long chain molecule is now put at 30,000 to 40,000. Stamm (52) obtained the latter value by centrifuging in a high speed Svedberg centrifuge. As the length of the chain varies, the molecular weight will vary.

There are many polymeric materials which are constituted on the same principle as cellulose in that their molecules are characterized by a chain of recruiting structural units; rubber is such a substance.

With this information as a starting point—though it was at the time less precise than now—the problem was carried forward by the X-ray workers (25, 29, 51). The spectrograms obtained indicate clearly that the structure of cellulose is symmetrical, that is to say, crystalline.

The long cellulose chains are aggregated into bundles of some sixty chains each. These bundles, being molecular aggregates, satisfy Nägeli's definition of a *micelle*. We shall recall that the botanist Nägeli postulated a so-called micellar structure for all gels, including protoplasm, the unit of the structure being a micelle or aggregate of molecules, i.e., a colloidal particle. As the cellulose micelle is symmetrical in structure and, therefore, crystalline, it has received the name of *crystallite*. An association of cellulose crystallites, oriented much as are bricks in a wall, presumably constitutes the colloidal structure of cellulose (45).

The precise orientation of the micelles is of significance in such properties as electric conductance, tensile strength, and elasticity. Mark (25) depicts two extremes, one in which there is perfect parallelism, and one in which there is a random or brush-heap distribution of the micelles, the former represented by native ramie and the latter by cellophane. The cellulose of flax displays an

excellent orientation of micelles parallel to the fiber axis, and has a tensile strength comparable to the best steel.

Carothers (5) adds another possibility, namely, that of pronounced overlapping of the molecules of one bundle with those of another, a very likely condition in that the molecular chains of a cellulose micelle are of different lengths. Such an arrangement would provide maximum strength in the direction of the fiber axis, because the mutual cohesive force of the long chains would be fully utilized. In regeneration cellulose (cellophane), says Carothers, there is random orientation. The molecules are brought into an ordered arrangement by mechanical stress. The strength of a sheet of cellophane which is initially the same in all directions, can be so changed by stretching that its strength along the axis of stretch is increased several times.

If we turn for a moment to other substances of an organic nature which have been subjected to X-ray study and found to be crystalline in nature, with linear units in often orderly arrangement, we find that the list is a long one; it includes starch, gelatin, chitin, rubber, silk, hair, keratin, sinew, muscle, nerve, and brain. It is but a step from these to protoplasm; indeed, muscle, nerve, and brain are protoplasm.

Frey-Wyssling (17, 18) has carried out extensive polarization studies on the cell walls of plants. The method is that of immersion in liquids of known index of refraction, and observation through Nicol prisms. By this means he showed that in cell walls there are submicroscopical (colloidal) rod-shaped particles which he identifies with the Nägeli micelles. The long axis of each micelle corresponds to the direction of the greatest refractive index; the latter value, therefore, gives the orientation of the micelles in the wall.

Photographs of cellulose taken with the Spierer lens add further evidence to the general conclusion that cellulose possesses a colloidal structure of symmetrically arranged rods (43). The lens reveals parallel striae which appear to be composed of microscopic units or *super-micelles*, oriented end to end. The striae form lamellae or plates which in their turn combine to produce the mass of cellulose. The same striated and articulate structure persists in bituminous coal as shown by Thiessen (53).



Question has arisen over the superficially similar structure shown by the Spierer lens in protoplasm and in cellulose. In both cases the structure is that of short rods, linearly oriented in parallel striae. That this confusion should arise is understandable, but the resemblance is purely superficial. If rolling country adjoining the sea, where large waves for the moment exist, is viewed from a distance, both land and sea would present the picture of parallel ridges, yet the material of which they are made and the forces responsible for their existence are entirely different in the two cases. So it is with protoplasm and cellulose in their finer microscopic structure. The punctated striae in the case of cellulose are built of oriented short rods, or super-micelles, of solid material. The punctated striae in the case of protoplasm are built of distorted, rod-shaped, liquid droplets, the dispersed phase of an emulsion under tension.

The exceedingly delicate macroscopic fibers of which wood is composed are built ultramicroscopic and molecular fibrils, such as those to which we have referred. The wood fibers in their turn build up the larger fibers characteristic of plan cellulose, e.g., cotton fibers. Natural cellulose thus consists of units of ever increasing size, all of which, from chain molecules to visible wood fibers, are of linear form. The orientation of these units determines the physical properties (elasticity, tensile strength, etc.) of the material.

Probably no other force in nature is so widely distributed and plays so great a rôle in the behavior of systems, from molecules to organisms, as does polarity. The term expresses any situation where the two ends or sides of an object are different, but in the strict chemical sense polarity should be limited to objects the ends of which are electrically unsymmetrical. Given long and polar molecules, molecules with ends electrically dissimilar, it is possible to picture their orientation in mass and to obtain a type of structure which is presumably typical of gels, and which at least has the virtue of giving a mechanical basis upon which to interpret the behavior of gels. Any linear molecule with unsatisfied terminal or lateral bonds, such as amino acids with ionized  $\text{NH}_3^+$  and  $\text{COO}^-$  groups or protein molecules with side chains, presents the possibilities of weak unions along the main chain. So-called internal salt

formation in proteins, and the many examples of tautomeric shifts, are similar cases. Such a situation meets the structural requirements of a brush-heap of loose construction, capable of constant readjustment. It is well illustrated in gels which exhibit the phenomenon known as thixotropy (15) which is a very typical property of protoplasm. The term refers to the sudden collapse of a gel from a firm body to a thin fluid as a result of mere mechanical disturbance. In ideal thixotropic systems, ferric oxide sol, bentonite, etc., the gel is reformed, again and again. Possibly all sudden changes in protoplasmic consistency, if not also muscular action, are instances of thixotropic change.

Viscosity measurements of protoplasm (40) have played a prominent part in protoplasmic structure, not so much because of the values obtained, but rather because of the discrepancy in values. Among the reasons for this discrepancy is the non-Newtonian (anomalous) behavior of protoplasm. It does not exhibit true viscous flow. This is denied by some workers who believe protoplasm to be a true solution with no "yield value" such as is characteristic of colloidal lyophilic solutions. Other reasons which may explain the divergence in values of protoplasmic consistency are methods in measuring, failure to realize that protoplasm undergoes very rapid changes in viscosity, and that, with possible rare exceptions, all parts of the cell are not of the same consistency.

Many workers have studied protoplasmic consistency because of its important bearing on physiological reactions, such as protoplasmic streaming, amoeboid movement, metabolic activity and muscular contraction. Where the change in viscosity is exceedingly rapid, thixotropy, rather than a simple viscosity change, is probably responsible.

Pure liquids, e.g. glycerine, and pure solutions have one viscosity value at all pressures. Lyophilic colloidal solutions, e.g. a gelatine sol, have different viscosity values at every pressure at which they are measured. They are said, therefore, to be non-Newtonian, because Newton's law of viscous flow does not apply. The fact that they deviate from this and Poiseuille's laws, indicates that there are structural features which interfere with pure viscous flow. The non-Newtonian or anomalous behavior of colloidal solutions is one of the best indicators we have of a continuity in structure. All such substances, proteins, etc., possess a *yield value*, i.e., they re-



quire the application of a force to start flow. If they lack a yield value they are Newtonian and show true viscous flow. The single constant laws of Newton, Maxwell, and Hooke do not apply rigidly to non-Newtonian systems nor do the laws of Stoke and Poiseuille. As protoplasm is elastic, exhibits thixotropic behavior, and contains a high proportion of protein, it is inconceivable that it should show true viscous flow. It will approach this latter condition when thin.

A number of apparently inconsistent results pertaining to protoplasmic consistency are capable of interpretation on a structural basis. One worker, by observing the Brownian movement of particles, obtains a low value for the viscosity of protoplasm. The particles may be in minute vacuoles (24) and the values, therefore, applicable only to the fluid aqueous medium within and not to the protoplasmic mass as a whole. Osmotic measurements, which suggest that protoplasm is a true solution, may apply only to the aqueous dispersion medium which bathes the protoplasmic framework. Such observations tell nothing of the structural features of protoplasm as an entity, of those features which are necessary to account for thixotropic behavior, elastic qualities, and immiscibility in water. Protoplasm *imbibes*, i.e., takes up, water; it does not *dissolve* in water; this implies structural continuity. Such properties of protoplasm are the best criteria we have of protoplasmic structure.

Scarth (36) says that protoplasm is characteristically elastic and the impression of fluidity is illusory. He cites the case of active streaming in freely suspended protoplasmic strands, which is possible only if there is a structural framework. Spek (46) is of the same opinion. So apparently is also E. B. Wilson (55) when he states that the "continuous substance" is the most constant and active element of protoplasm and forms the structural basis of the system. E. G. Conklin (9) adds that protoplasm is composed of a more fluid and a more viscid portion. He bases his statement on experiments in centrifuging the eggs of *Crepidula* where he found that the more fluid portion of protoplasm may be readily moved but the more viscid portion is not so readily moved; the more viscid part of the protoplasm holds the nucleus in definite relation to the periphery of the cell and brings parts back to their normal positions when once they have been displaced by centrifuging.

Another property which is typical of elastic jellies is that of *syneresis*, or the squeezing out of some of the aqueous medium by a slow contraction of the gel. The property is nicely exhibited in protoplasm, being most pronounced when abnormal conditions arise.

If we now list those properties of protoplasm which force us to recognize it to be essentially a jelly, a lyophilic colloidal system, whether firm or fluid, they are: elasticity, rigidity, extensibility, imbibition, water-immiscibility, thixotropy, syneresis, and coagulation.

One of the obstacles to the universal acceptance of structural continuity in protoplasm has been the notion that a framework is inconsistent with the evident fact that protoplasm flows. So do thixotropic solutions flow, but they immediately build up again into firm gels. The framework of protoplasm is constantly changing. The linear structural units undergo continuous readjustment, due possibly to tautomeric shifts.

It is significant for the problem of protoplasmic structure to realize that while polarization studies of protoplasm have not shown living matter generally to be anisotropic yet they have shown striated muscle, types of connective tissue generally, and chlorophyll to be anisotropic. Furthermore, muscle, nerve, and brain, which are protoplasm, have yielded spectrograms (X-ray diffraction patterns) as have sinew, hair, silk, etc., indicative of a crystalline nature.

Support for linear units in protoplasm comes from a considerable diversity of observations. Fibrous structures are typical of fixed cells. Living protoplasm is often of a "stringy" appearance and is of high tensile strength. Protoplasmic strands may snap with great suddenness and recoil. Muscle is fibrous (13). Nerve tissue is a bundle of threads. A. R. Moore (30) finds that plasmodia when forced through moderately fine sieves do not live, but they may of themselves flow through exceedingly fine sieves. Forcing crushes the long protoplasmic fibers, while in flowing naturally the protoplasm can take the fibers through very fine pores. Moore believes the microfibrils to be of the order of  $5 \times 10^{-5}$  mm. in diameter and 2,000 times as long. Peters (33) has postulated similar but finer molecular threads in protoplasm. Needham (32) discusses the importance of the problem.

The possible significance of cytoplasmic structure in physiological behavior is indicated by A. R. Moore (31) who finds that neither sperm nor egg nucleus of echinoderms has any effect on segmentation tempo, the reactions of the cytoplasm alone determining it.

The eternal question of how it is possible for protoplasm to carry on so many different processes simultaneously, without one interfering with the other, within the confines of a single cell, may be answered by the justifiable supposition that delicate membranes, consisting of nothing more than firm protoplasm, traverse the cell in all directions. An excellent example of this is to be had in a myxomycete plasmodium where there are set up temporary channels or arteries of protoplasmic flow. These arteries guide the protoplasm along definite routes which are broken down and re-established as the plasmodium progresses. The streaming protoplasm does not pass beyond the ephemeral boundaries of the arteries, though the latter are also of protoplasm. Their formation and temporary maintenance are undoubtedly made possible by a structural (fibrous) framework which endows the membranes with the required degree of rigidity.

*Protoplasmic organization.*

Whatever life may be and however much we may try to explain it on the basis of relatively simple phenomena, there always remains that greatest of all bodily and protoplasmic qualities, *organization*. To fully interpret cellular or protoplasmic organization in physical terms is, in the present state of our knowledge, impossible. The living system is too intricate; it is life itself. We believe, however, that the most fundamental characteristic of organization is structure. A study of chemical constitution alone will go no further in revealing the mechanism of even the simplest processes in protoplasm than it has in non-living systems. Structure, as well as chemical constitution, and the dynamics resulting from both, are necessary. The structure responsible for protoplasmic organization and most other physical properties of protoplasm, is a continuous but labile framework. Life in a discontinuous system is inconceivable. Aggregation, not dispersion, is the rule in living, as it is in non-living, colloidal phenomena. The harmonious functioning of a cell, which is but another name for life, is possible only because of the structural continuity of protoplasm.

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# THE BOTANICAL REVIEW

VOL. I

FEBRUARY, 1935

No. 2

## GLACIAL AND POSTGLACIAL VEGETATION\*

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To reconstruct the vegetation of the past involves, as does any historical research, numerous sources of error and omission. Yet reconstruction is necessary if we are to understand the changing environment of the past or to interpret modern vegetation. There is no escape from considerable, legitimate, inference based on geology as well as upon living plants and communities believed to be relicts of past conditions. But so far as possible, inference must be checked by direct evidence in the form of organic remains whose sequence and identity can be verified. In this connection pollen preserved in peat and related deposits is especially useful; and because of the close association of such deposits with glaciation they have been much employed in the study of glacial and postglacial vegetation. The results so obtained form the main basis for the following discussion which will deal first with the Pleistocene,<sup>1</sup> then with postglacial conditions.

On the basis of what is known of the various glacial limits and the present temperature zonation of plant life there has been considerable conjecture as to the pattern of vegetation at the times of maximum ice advance. The absence of barriers to the south and west in North America afforded a condition which does not obtain in Europe where the retreat of plants away from the ice was restricted. The general theory is that tundra, scrub, conifers and deciduous forest shifted as belts before the advancing ice, then followed back in its wake as it melted. Actually, of course, the ice

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<sup>1</sup> The Pleistocene was the great Glacial Age during which time ice extended over 8,000,000 sq. m. of Europe and North America, and great areas of Asia and the southern hemisphere. Estimates of the duration of this period range from 500,000 years to two or three times that figure. The retreat of the ice from Sweden has been placed at 12,000 years ago and from the northern United States at least 25,000 years ago.—Editor.



lobes extended into the interior grasslands as well as into the forest regions. Moreover, the exact character of the change of climate which produced glaciation is not proved. Certainly the mere proximity of glacial ice is not enough in itself to prevent the growth of trees. Trees ferns are found within sight of the New Zealand glaciers. Spruce-fir forests develop almost at the edge of the retreating ice in Alaska and there is some evidence to show that in places the ice has advanced over standing, possibly living forests (11). In the north central states it has been difficult to find any trace of tundra plants at the base of the oldest living deposits examined, a fact which will be discussed later.

On the other hand, Piech (38) states definitely that the crest of the Riss (III) ice sheet was fronted by a strip of treeless tundra more than 100 km. wide and Firbas (19) reports similar conditions nearly 450 km. ahead of the Würm (IV) glaciation. In the Florida peat deposits Hanna finds diatoms which he considers to have been deposited during the climax of the last glaciation (27). The species are so definitely northern in character that he considers the evidence "overwhelmingly in favor of cooling." Many of the forms now occur no farther south than New England. This leads him to consider the warm Pleistocene flora found by Berry in North Carolina to be interglacial or at most intraglacial, rather than a proof that the influence of the ice did not extend far south of its limits.

The whole problem of displacement of vegetation at the maximum advance of the ice is so significant that it warrants a careful study of all of the scattered and discontinuous peat deposits south of the glacial limits. The task is enormous but it seems the best means of deciding whether glaciation was accompanied by a general and extensive lowering of temperature. Evidently snow limits were lower in tropical mountains during the Pleistocene than now and many of today's deserts were then humid. But we do not know how the latitudinal limits of the various zones beyond the ice edge were affected.

Passing from the question of glacial to that of interglacial climate, we are confronted with an embarrassing confusion as to the number, character and correlation of these interglacial stages. Opinion, even today, ranges from the belief that only one such in-



terval can be dignified by the term interglacial, all other periods of recession being intraglacial, to the belief that five or more truly interglacial intervals existed during the Pleistocene.

An excellent idea of the situation may be obtained by realizing that the Toronto interglacial beds have at different times been attributed to at least four different intervals. Of the Aftonian interval Thwaites remarks, "strangely enough, although the evidence on which the Aftonian was originally defined is worthless there is, nevertheless, a real 'Aftonian' interval of great length and possibly constituting a genuine interglacial stage" (45).

There is a growing tendency to emphasize four major glaciations during the Pleistocene. Brückner (7), with Leverett, regards them as essentially contemporaneous in Europe and North America, just as students of lesser climatic fluctuations of recent times point to a certain correlative behavior throughout the northern hemisphere. Disregarding subglaciations the scheme is as follows:

<i>Period</i>	<i>North America</i>	<i>Europe</i>
IV	Wisconsin	Würm
III-IV	Sangamon (Interglacial)	Riss-Würm
III	Illinoian	Riss
II-III	Yarmouth (Interglacial)	Mindel-Riss
II	Kansan	Mindel
I-II	Aftonian (Interglacial)	Gunz-Mindel
I	Nebraskan	Gunz

As to subglaciations there is still much discussion. The Iowan substage is important because of its well marked interval, the Peorian. Leverett apparently still regards these as late Illinoian, but Kay in Iowa and Leighton in Illinois have recently agreed in placing them in the early Wisconsin, thus following rather than preceding the Sangamon interglacial (8). Similarly in Europe there is some disagreement as to the position of the Warthe subglacial as indicated by Firbas and Grahmann (21) and Premick and Piech (38).

Szafer (44) in 1925 presented an account of the Polish interglacial periods in which he ascribed prolonged warm conditions, partly oceanic, partly continental to the last interglacial (Riss-Würm, Masovien-II). Jessen and Milthers (32), working in Jutland and northwest Germany, agree with him, describing the last or Riss-Würm interglacial as passing up to an atlantic (warm, humid)

climax and back, with a brief temperate fluctuation during the cool closing period. The penultimate (Mindel-Riss?) is described as simpler, progressing up to a warm climax and then back to glacial conditions.

However, all of the sites except one which were described by Szafer as Riss-Würm (Masovien II) have now been correlated by Premick and Piech (38) with Mindel-Riss (Masovien I). In a detailed analysis they show the latter to have been of more marked character than the former, or last interglacial. Four beds of Mindel-Riss age show gradual progress from pine-birch through oak, then oak-linden-hornbeam to fir, fir-spruce, and back to pine. During this final pine stage there appears to have been a brief amelioration marked by temporary increase in oak and other hardwoods. These quickly vanished before pine, arctic birch and willow, heralds of the returning ice. A similar analysis of the last interglacial agrees in its general import but differs in detail, presenting a predominantly cool and moderately humid character. It appears to have been mainly coniferous with briefer and weaker expressions of the mixed oak phase.

Dokturowsky (14) has found similar conditions for the last interglacial in Russia. Following the Riss-ice was a cold period with pine, birch and willow. This was succeeded by a warm period with hazel, alder and mixed oak forest, while another cold period with spruce and fir preceded the Würm glaciation. Dokturowsky is unable to confirm Szafer's report of a prolonged and intricate character for the last interglacial. With respect to the preceding, interglacial information from Russia is scanty, remains of yew, beech, larch and *Trapa* appearing to comprise the finds reported. Brookes, with others, considers the penultimate or Mindel-Riss as of much greater length and more marked character than the other interglacial periods, and states that it should probably be correlated with the prolonged Yarmouth stage in North America.

Firbas and Grahmann (21) report that deposits following the Warthe subglaciation in south Brandenburg show fluctuation between birch and pine, the latter accompanied by traces of deciduous forest. The period appears to have been predominantly cool. Spruce appears, although afterwards completely absent from the late glacial and early postglacial stages in the same vicinity.

In addition to these more comprehensive recent studies there is a considerable number having to do with isolated and fragmentary profiles, correlated with varying degrees of certainty. From all of the work to date, incomplete as it is, certain principles appear clearly enough. There is a general and significant, even if not detailed, parallel between the events of postglacial time and those of the interglacial intervals, so far as the latter are known. In the second place there was a sufficient variety of mild climates during one or more of the intervals to have permitted extensive migrations of plants, including some which had been thought of as tertiary rather than as interpleistocene relicts (44).

For North America, the contents of the Toronto beds show that during whatever interglacial period they were laid down, vegetation flourished, which suggests a milder climate than that of Toronto today (10). Toronto is within but near the present northern range limit of the linden and papaw, while the natural range of the Osage orange is distinctly restricted to the southwestern Mississippi Valley.

Evidence of a marked interglacial climate during what Kay considers the Aftonian, or first interval, has been secured by Lane working with the Iowa Geological Survey. His results have not yet been published but the writer has examined, and in some cases checked, his material. Acknowledgment is here made for courteous permission to refer to this and other data in advance of publication. Lane finds evidence in the Denison beds of a prolonged steppe or grassland period which passes through a brief deciduous forest phase into coniferous forest.

Two independent studies have been made on the Sangamon, an unpublished one by Lane on the Wapello beds in Iowa, the other by Voss (47) in Illinois. The results of both studies are in substantial agreement. Voss reports vegetation indicating conditions in Illinois similar to those of the conifer belt of the northern states and southern Canada. Lane finds conifers throughout. Pine with grass, oak and spruce later yields to a cover of pine and spruce. The Wapello beds are considered late Sangamon.

The Peorian, now considered intraglacial, whether within the late Illinoian or early Wisconsin, has also been studied by Voss, with findings of a conifer climate similar to that described by him for the Sangamon. The climatic resemblance of the Sangamon to

the Peorian is much more consistent with the recent reports from Poland regarding the Riss-Würm than with the earlier report of Szafer or the findings of Jessen and Milthers in Jutland and north-west Germany.

In addition to the report of Voss on the Peorian, we have reports on intraglacial beds of late Wisconsin age from Wilson (49) in Manitowoc County, Wis., and from Cooper and Foote (12) in Minneapolis. Both agree that the organisms found indicate conditions now prevalent to the north of the sites investigated, thus confirming their intraglacial character.

Before leaving the subject of climate during the glacial period, it seems advisable to mention the importance of the loess. This wind deposited soil is considered an excellent criterion of dry conditions. Several occurrences of it have been correlated as interglacial and so used to predicate marked continental conditions during those intervals. This assumption is strengthened, not only from the character and mode of origin of the loess itself, but from the character of the molluscan remains which it shows. Baker (4) has studied the latter in loess deposits of the Yarmouth, Sangamon, Peorian and two Wisconsin intraglacial periods. In addition to several extinct species he finds others whose present range is definitely western or northern. If practicable it would be of interest to see the same statistical methods applied to molluscan remains which have proved so useful in the case of fossil pollen.

Five distinct soil horizons, separated by loess, have been described from Bermuda by Sayles (40). The soils have been attributed to mild humid inter-glacial conditions, while the loess is considered to be due to the severe, windy periods of glacial advance. Harper (28) has found somewhat similar conditions in Oklahoma which he ascribes to the Pleistocene. The Rocky Mountain region and the Southwest abound in fossil soils or humified layers separated by layers of aeolian material or heavy alluvial wash suggesting arid intervals, but their correlation is necessarily a difficult matter, requiring great caution.

The climate and vegetation of postglacial times has been much more thoroughly studied than have Pleistocene conditions. Nearly 100 years ago in the Danish peat bogs, Steenstrup found successive strata of buried forests not showing evidence of a steadily warming climate since glacial times. As decades went by, the study of

fossil remains in the peat and of the peat layers themselves suggested strongly that the climate of Europe had once been milder than today. This probability seemed to be increased by the presence of relict steppe plants in places having today a forest climate.

The solution of the problem was greatly complicated by historical, traditional and even stratigraphic evidence indicating climate fluctuations of considerable intensity but short duration. Moreover, the destruction of plant cover and humus following human occupation always tends to create an impression of greater aridity than the climate justified, except perhaps in areas having a marked oceanic climate. This, of course, confuses the issue and has doubtless been responsible for much of the prevalent notion that our present climate is becoming drier. That it is becoming cooler seems warranted by the cultural history of Iceland, the Esquimaux, and by the southward shift of the limits of the vine.

Hypothesis has passed through four important phases as follows:

First, the simple assumption that vegetation had moved south before the advancing ice and subsequently returned to a more or less stable equilibrium. This idea is still implicit in many quarters (24, 48).

Second, the Blytt-Sernander hypothesis, postulating a series of fluctuations in both moisture and temperature, and designating the following periods: pre-boreal, boreal, atlantic, sub-boreal, sub-atlantic. The atlantic or middle period was conceived of as a climatic optimum, warm and humid, between the very continental boreal and sub-boreal. The sub-atlantic represented a return of humidity. The stages so set forth were associated with a definite stratigraphy and archeology, and dated with great accuracy through the method of DeGeer (42). They thus afforded something very tangible, capable of being checked with most minute care throughout Europe. The value of this hypothesis as a scientific tool is not to be underestimated, even by those who have rejected it. As is often the case, it has probably suffered more from its friends than its enemies.

Third, G. Anderson (1), holding the view that temperature changes have been the predominant ones, conceded that glaciation was followed by a very dry and ultimately warm boreal period, succeeded in turn by an equally warm but much wetter atlantic. But he denied the reality of a second dry or sub-boreal period and

predicated a gradual climatic deterioration from the atlantic (neolithic) down to the present. In this general interpretation Gross (25) and Rudolph (39) appear to concur.

Fourth, L. von Post (46) divides postglacial time into three periods: 1., a period of increasing warmth; 2., a period of maximum temperature; 3., a period of decreasing temperature. On this general frame-work, he admits, lesser fluctuations of the various climatic factors have had their place. Under suitable conditions the minor fluctuations have doubtless been extreme enough to produce a marked effect. Very soundly, he concludes that only by the most minute analysis between correlated horizons, on a widely coordinated plan, can we determine the extent, character and intensity of the actual fluctuations. Gams (22) appears to agree essentially with von Post but considers that the waning of the warm continental maximum has occurred in oscillating fashion, thus explaining whatever evidence may exist for a dry sub-boreal about 3000 years ago. He also considers the evidence of recent drying not to be wholly due to human interference.

Having thus outlined the trends of theory, let us examine the phenomenology upon which they are based. The difficulty of accepting an hypothesis of return to stable climatic equilibrium following glaciation in Scandinavia has already been noted. The pioneer work of Steenstrup was followed by much more detailed study of bog profiles, plant fossils and molluscan remains, much of which went counter to the idea of a stable postglacial equilibrium. Both in Europe and North America taxonomists and students of floristics found themselves confronted with problems to be explained only by assuming a warm continental climax some time during the postglacial (23). Geological analysis of glacial retreat showed that it had been marked by periods of re-advance. Furthermore, the demonstration of huge-scale fluctuations, such as the Permian<sup>2</sup> and Pleistocene as well as the so-called "cycles" of small magnitude have, it must be confessed, placed scientists in a receptive mood toward the demonstration of fluctuations of an intermediate order, to be measured in tens of centuries perhaps.

<sup>2</sup> Permian time antedated the Pleistocene by great eons. It succeeded the Carboniferous as the last of the Paleozoic. In South Africa, India and Australia evidences of glaciation during this earlier epoch, too, have been found. During Permian time conifers probably made their first appearance on earth.  
—Editor.

Allowing for appropriate differences in alpine and other extreme habitats, the general vegetational history in Europe has been about as follows. Widespread and prolonged postglacial tundra conditions are indicated by remains of *Dryas*, birch and willow, far south of the ice front. It is generally found that this was succeeded by a period in which pine predominated, variously accompanied by birch and hazel. The pine was followed by oak, the hazel frequently persisting on into the oak period. In fact, hazel, considered to be an indicator of dry continental conditions, exhibits a singular behavior so far as its relative pollen frequency is concerned. Its maximum may precede, accompany or follow that of the oak. Perhaps as much as any other factor this contributes to the uncertainty as to whether conditions were dry during both the pine (boreal) and oak (sub-boreal) or only during the former. Erdtman (15), in a very penetrating analysis, points out certain contingencies which may affect preservation of the hazel pollen even when abundant, while Firbas (20) notes that weathering of peat during the hazel maximum may interfere with the study of its pollen content. In the words of Theophrastus, "this, then, is proper subject for further investigation."

The oak period, characterized by an admixture of other deciduous trees such as linden and elm, exhibits a variable length and intensity of expression. It is least marked at high altitudes and latitudes, more so in continental than oceanic areas. In a large part of Europe the oak appears to have been replaced eventually, either through an intermediate stage of beech or without it, by conifers, birch and alder, suggesting cooler and perhaps more humid conditions.

In addition to the changes of vegetation, as shown by pollen analysis, the changing character of the peat itself affords some evidence. In the late glacial stages peat formation seems to have been restricted and exceedingly slow which has prevented an adequate record of events and measure of time. With the establishment of a sub-arctic forest of birch and pine, heavy formation of sedge peat occurred. Later during the warm, dry (boreal) climax (hazel) this peat was subjected to heavy humification which often spoiled the record. During the mixed oak stage, formation was renewed (atlantic) but apparently often this was followed by another period of weathering (sub-boreal). On the latter point there is consid-



erable controversy between those who believe in a dry sub-boreal, and their opponents. The weathered sub-boreal (?) layer in question is known as the Grenz-horizont. According to Gross (26) its origin and even existence is frequently a matter of doubt. Above the mixed oak strata is found a renewal of peat formation, rather generally attributed to "climatic deterioration"—either lowered temperature, increased moisture or both. Quite generally the surface layers of peat show disturbance, attributed by many to cultural effect, but thought by others to be due to a recurrence of dryness.

There is apparent a growing and hopeful effort to isolate all of the factors such as migration rate of trees, late glacial interstages, local plant succession, soil development and climatic change. The reader interested in further information should consult the recent, more general papers of Gams (22), Gross (25), Firbas (19), Keller (34), von Post (46), Rudolph (39) and others cited by Erdtman (17).

By comparison with Europe, North American studies are still meagre and tentative. The area is vast and diversified with its own geological history. The absence of marked barriers to plant migration and a decidedly richer forest flora than that of Europe introduce complications. Although there is a growing belief in the similarity of climatic trends, it is essential that the American problem be approached from a strictly objective basis.

As in Europe, the known peat record does not extend back continuously beyond a maximum of about 10,000 years. Such late-glacial records as are known appear to be isolated and to indicate general northerly conditions, one from the Connecticut valley being pronounced by Hollick (29) to be the American equivalent of the European *Dryas* flora. There is not much but conjecture as to conditions beyond the ice-front, although Hanna's (27) report of diatoms in northern Florida which now range from New England northward is significant.

There appears to be little or no record of tundra at the bottom of living peat profiles. Instead, the record begins with a marked abundance of fir and spruce pollen, not only in southeastern Canada (3) and the Lake States (41) but even in Iowa (35). This does not preclude the possibility of a long tundra stage. The basins suitable to peat formation may have been occupied by dead ice until the forest returned northward to surround them. Tundra



may possibly have been followed by steppe conditions so dry as to preclude peat formation. Or, as in Alberta (16), the central states may have been covered with an aspen parkland and yet have shown a pollen profile in which only conifers were indicated. Antevs (2), in common with many others, believes that a tundra zone existed but that it may have been comparatively narrow. Further study may elucidate this point.

While the bottom of the record in eastern North America shows the general presence of spruce and fir, above that point important regional differences appear. In southeastern Canada (3), Minnesota and Northern Wisconsin (48) pine quickly replaces the other two coniferous genera and is predominant until the upper layers where spruce and fir once more increase at the expense of pine. Whatever indications of deciduous trees there may be, they are strongest during the middle of the pine period, not inconsistent with the idea of warmer conditions than now obtain. In Canada, hemlock shows a slight but definite tendency to appear before and after the pine, rather than with it. This suggests to Auer that the pine corresponds to the European sub-boreal, preceded and followed by more humid periods, the atlantic and sub-atlantic, respectively. On the other hand, Voss reads no such meaning into his own profiles from the north lake states although he concedes their similarity to those of Auer. He attributes the recent increase of spruce to local conditions without discussing their striking coincidence with local effects across half of this continent, not to mention northern Europe and Japan (33).

Profiles from central Wisconsin (48) and in less detail from Michigan (37) and northern Indiana (31, 36) resemble in their general character those just described save that there is a definite interval of deciduous predominance instead of a mere showing during the pine period. To varying degrees these profiles show indications of a recent increase of conifers at the expense of deciduous genera, again suggesting the passing of warmer conditions.

In Ohio (41), central Indiana (31) and northern Illinois (48), following the conifers, the deciduous interval is marked and prolonged through to the present. In northern Illinois, where it occupies the upper two thirds of the profile, Voss states that climate has been essentially uniform and attributes any fluctuations to edaphic and topographic causes. In Ohio the fir and spruce are first re-

placed by pine. The deciduous forest interval then begins with an appearance of white pine, beech and hemlock, later showing a predominance of oak-hickory with a recent and slight but definite increase in beech. Sears has interpreted the oak-hickory period as dryer, perhaps warmer than those which preceded and followed it. The transition from coniferous to deciduous he considers to be moister than the pine period which preceded it.

In Iowa (35) the coniferous stage passed through one of deciduous forest into grassland near the base of the profile. The grassland shows at least two periods of abundance of pollen, indicating semi-arid conditions. Sears has correlated these tentatively with the pine and oak-hickory periods in Ohio, and the latter with Auer's pine period in Canada.

It must be conceded that any correlations at the present time are highly provisional and that an immense amount of work needs to be done. The first task is to establish the broad outlines of major change. A simple approach should be to test whether von Post's hypothesis of increasing warmth, warm maximum, and decreasing warmth is justified as it seems to be by much of the work except that in Illinois. In a transitional region like Illinois it is essential that search be made for strata showing grass pollen and that species, as well as genera, having a complementary character be studied. In Ohio this was done by using beech and hickory. Hotchkiss and Ingersoll (30) report geothermal measurements from the Calumet district which agree with the requirements of von Post's outline. Incidentally, Dachnowski (13) reports petrographic sequences in his peat profiles such as would be expected if not only the general hypothesis of von Post but the moisture fluctuations postulated by Auer and Sears were a reality. Certainly these assumptions all need much more rigorous proof than they have at present. Complications may be expected because of human disturbance of recent vegetation and the possibility, however remote, that there has also been a recent swing towards drier climatic conditions. Furthermore, it is essential to reckon with the probability that climatic shifts, if any, "have not operated uniformly nor persisted equally in all types of habitat" (42).

In the meantime, there is a healthy enough divergence of viewpoint among American students to insure that any hypothesis whatsoever will be thoroughly tested, including the hypothesis that

much of postglacial climate has been uniform (3, 6, 9, 13, 24, 42, 48).

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## THE STRUCTURE OF THE WALLS OF THE HIGHER PLANTS

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The presence of a definite more or less rigid wall enclosing the protoplasm is one of the important characteristics of plant cells, but the structure and chemical composition of this wall varies enormously when the entire plant kingdom is considered. The origin of these conspicuous cellular membranes, the details of their structure and the changes that they undergo during the growth of the organism have interested botanists for nearly a century. It is interesting to note, however, that the structure of the plant cell-wall can no longer be regarded as a problem of strictly botanical interest. The wide use of cellulose in modern industry has focussed the attention of workers in many different fields upon the problems of cell-wall structure. Papers dealing with these problems may be found about as frequently in the professional journals of the chemist and physicist as in those of the botanist. The development of new methods of investigation has made it possible to discover structural units in the wall that are far beyond the range of the finest modern microscopes. Not only can the existence of these sub-microscopic units be detected but their spatial arrangement in the wall can be determined with some precision and the changes that they undergo during the development of the wall can be studied. Dependable information regarding the molecular orientation of substances composing the structures of the cell-wall has seemed, until recently, far beyond the limits of human ingenuity. It is now, however, definitely within the scope of experimental study and entirely possible for the botanist to obtain very concrete pictures of the spatial arrangement of the molecular groups that make up the cell-wall as well as of the orientation of the various atoms that make up these molecular groups. Before undertaking a discussion of these recent developments it will be desirable to sketch briefly the evolution of ideas regarding plant cell-wall structure.

The prominence of the plant cell-wall led to the discovery of the cellular structure of plants. It was only natural that early observers should have been impressed with the cell-wall and formed

exaggerated ideas regarding its rôle in vital activity. The discovery of protoplasm and the gradual realization of its fundamental significance transferred interest from the conspicuous cell-wall to the contents of the cell. The wall lost its position of preëminence in the minds of investigators and became generally considered as a passive non-living membrane surrounding the active protoplasm. This idea of the wall as being sharply and distinctly separated from the living protoplasm, as being a dead non-living material produced by protoplasmic activity, has persisted until very recent time.

The wall of the plant cell is not homogeneous but shows definite lamellae when viewed in cross-section. These lamellae usually differ in their refractive indices and, consequently, appear with different degrees of brightness. In addition to this stratification within the wall, surface markings of various kinds, especially spiral striations, are frequently visible. These structural features of the wall become more conspicuous when the wall is swollen with suitable reagents and they decrease in visibility as the wall is dehydrated. When cross-sections of cell-walls are viewed between crossed-Nicols<sup>1</sup> certain lamellae in the wall appear dark, i.e., isotropic, while other lamellae show varying degrees of double refraction.

These facts led Nägeli (39), who made the first extensive study of cell-wall structure, to suggest that the wall was composed of submicroscopic units or *micellae* arranged in definite layers. Each micelle was believed to be surrounded by a layer of water and so separated somewhat from adjacent micelles. The stratification visible in cross-section was explained by assuming a difference in the size of the micelles and, therefore, a difference in the amount of the adsorbed water. Stratification was, accordingly, believed to be the result of micellar layers of material with relatively large amounts of adsorbed water alternating with layers of micelles with smaller amounts of adsorbed water. Differences in the amount of swelling along different cell axes were explained by assuming the micelles to be elongated. Cubical micelles would have equal amounts of adsorbed water on all sides while elongated micelles would form a membrane having more adsorbed water in one axis than in another. This micellar theory of Nägeli has been the ob-

<sup>1</sup> For definition of crossed-Nicols see Gage, S. H., The Microscope.—Editor.



ject of considerable discussion and debate for nearly three quarters of a century. The most modern methods of cell-wall investigation have only served to emphasize its essential accuracy and today it is probably more widely accepted than any other theory of cell-wall structure.

Early investigators discovered the fact that various lamellae in the wall reacted differently to dyes. Certain lamellae stained intensely with specific dyes while others stained only slightly or not at all. This phenomenon was rightly interpreted to indicate the presence of chemically different materials in the wall. The existence of chemically different materials in the wall was also shown by the action of various solvents.

The most important constituent of the wall is cellulose since it forms the skeletal framework of most plant cell-walls. Associated with cellulose in the cell-wall are five other common membrane substances: *lignin*, an amorphous material, generally associated with cellulose in woody tissue; *pectic compounds*, substances of colloidal character that make up the original middle lamella and occur with cellulose in most plant cell-walls; *hemicelluloses*, wall compounds that are polymers of pentose and hexose sugars and that can be hydrolyzed with hot dilute acids; *cutin* and *suberin*, compounds with lipoid properties that occur in epidermal cell-walls, cork tissue and in a few highly specialized cell-walls. In addition to these membrane materials the cell-wall may contain greater or lesser amounts of minerals and organic compounds that play no essential part in its structure.

#### *The Origin of the Cell-Wall*

The exact mechanism by which the cell-wall originates in the higher plants is not definitely known. The new cell-wall is usually formed after nuclear division. A protoplasmic plate, the *cell-plate*, forms between the daughter nuclei. Early investigations led to the belief that the cell-plate was formed from the spindle fibers but the investigations of Bailey (4) and others (46) indicate that the cell-plate may continue to develop after nuclear division is complete. Two views of the formation of the first wall are current: (a) the cell-plate divides longitudinally and the new wall is deposited in the cleft between the two halves of the cell-plate; (b) the cell-plate itself becomes transformed into the material of the



wall either through a change in its composition or as a result of the addition of new material. It seems possible, in the light of recent work as discussed by Sharp (46), that the cell-plate of the earlier workers is an artifact produced by the fixing agents used. Recent work (46) indicates that the region between the daughter nuclei is actually more liquid than the adjacent protoplasm and that membrane materials are deposited in this liquid zone forming the first wall between the daughter nuclei. There is abundant evidence that the cell-plate develops independently of the spindle fibers between the daughter nuclei and that a large proportion of this first wall is formed by the growth and extension of the terminal portions of the cell-plate itself.

However the first wall between the cells may be formed it is known to be composed of pectic material. To this pectic material between the protoplasts of the adjacent cells, a layer of cellulose containing pectic material is subsequently added by each protoplast. This first cellulose layer forms the primary wall. It differs in several essentials from subsequent layers of cellulose that may be added to this membrane during the thickening of the wall. The primary wall is capable of growth and enlargement and of undergoing reversible changes in thickness (29). The secondary wall consists of all the subsequent cell-wall material deposited within the primary wall. This material is usually added in the form of definite lamellae which, as a rule, are not identical in their physical and chemical properties. Because of these differences in chemical or physical properties the secondary wall itself is sometimes subdivided. This interpretation seems unwise in view of the fact that the secondary walls of different cells differ greatly in their structure and additional subdivisions of the cell-wall into tertiary or quarternary deposits could have no general application or any uniform morphological significance.

The terms *middle lamella*, *primary wall* and *secondary wall* have not been used by different investigators to apply to the same structural portions of the cell-wall. This has led to considerable confusion and it is necessary for each worker to define his use of the terms. In an effort to clarify this situation and to bring some order out of the present confusion of cell-wall terminology, Kerr and Bailey (29) have carefully reinvestigated the subject and proposed the following use of terms:

1. *Middle lamella* or *Intercellular material* as the amorphous, isotropic<sup>2</sup> material largely, if not entirely, pectic compounds first deposited by the cytoplasm.

2. *Primary wall* or *cambial wall* as the first anisotropic<sup>2</sup> layer of the wall composed largely of cellulose and pectic materials. It is capable of growth and extension and has the capacity for undergoing reversible changes in thickness.

3. *The Secondary Wall* as the additional wall layers formed within the primary wall. Cells having secondary walls lose their potentiality for growth and enlargement.

In their study of the first cell-walls formed, Kerr and Bailey point out that the so-called middle lamella of earlier workers included at least three distinct layers of the wall: an isotropic inter-

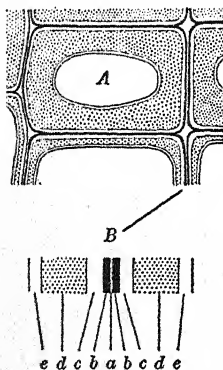


FIG. 1. (From Bailey). (A). Diagrammatic cross-section of a tracheid illustrating the various layers of the wall. (B). Enlarged section of the wall illustrating the terminology applied to the different layers of the wall: (a) middle lamella or intercellular substance, (b) primary walls, (c, d, and e) layers of the secondary wall.

<sup>2</sup> Isotropic substances appear dark when viewed through crossed-Nicols. Anisotropic substances are visible through crossed-Nicols, assuming different positions in response to light.—Editor.

cellular material and two anisotropic layers containing cellulose (the two primary walls). The greater intensity of the double refraction in the first layers of the secondary wall overshadows that of the primary walls which is relatively faint, possibly because of a higher content of colloidal material of pectic nature. Fig. 1 taken from their paper makes clear the layers of the wall that the proposed terms will designate. It is to be hoped that this terminology will be generally accepted so that the terms *middle lamella*, *primary wall* and *secondary wall* may be used by future workers without danger of being misunderstood.

#### *Methods Available for Studying the Structure of the Cell-Wall*

The problem of cell-wall structure has been attacked in a number of different ways. Eight or ten different lines of investigation have been used with success and each has added its contribution to the final picture. The modern conception of cell-wall

structure is really a mosaic carefully built from the evidence slowly acquired from various experimental methods. The contributions that each of these methods has made will be briefly outlined.

1. *Direct Observation of Untreated Material.* A great deal may be learned about the structure of the plant cell-wall by merely examining sections cut in different directions. The existence of definite layers in the wall is at once apparent. Surface markings such as striations, radial lines, pits and irregular thickenings frequently suggest the existence of fundamental structural divisions. Further, differences in refractive indices of the different layers indicate the presence of different materials.

2. *Differential Staining.* The existence of specific membrane compounds may be indicated by differential adsorption of certain dyes or by color reactions resulting from specific chemical changes (Plate Fig. 1). Differential staining is an important tool in cell-wall studies if properly used but it is not infallible. Differential adsorption is rarely complete and is markedly influenced by the concentration of the dye, the acidity of the medium, the solvent used and by the presence of unknown substances in the membrane. The experienced worker can gain much information regarding the presence and localization of certain membrane constituents from staining reactions. The recent extensive studies of Czaja (10, 11) upon metachromatic staining indicate that differential staining may also give information regarding the spatial distribution of the submicroscopic units in the wall. Kisser (30) has also shown that physical differences in the wall may cause beautiful examples of the differential adsorption of dyes.

3. *Differential Solubilities.* The presence of different chemical compounds in the wall of the plant cell can be nicely shown through differences in the solubilities of these materials in certain reagents. Copper-oxide-ammonia, for example, will remove unmodified cellulose from the wall without removing cutin, suberin, lignin or pectic materials. Similarly, each of the mentioned wall constituents may be extracted from the wall through the use of suitable solvents. The importance of this method of investigation is illustrated in Anderson's (1, 2, 3) study of collenchyma and epidermal cell-walls, by Lüdtke's (36) work upon fiber cells and by Kerr and Bailey's (29) interesting investigation of the middle lamella. (Plate Figs. 2 and 4).

4. *Polarized Light.* The possibilities of polarized light as a method of investigating the structure of the cell-wall have been well shown in the extensive studies of Frey-Wyssling (17, 18, 19, 20, 28). Polarized light not only permits the detection in the cell-wall of isotropic materials such as pectic compounds and lignin and the localization of anisotropic constituents as cellulose, hemicellulose and cutin, but also furnishes information regarding the spatial orientation of the submicroscopic units that make up the cellulose wall (Plate Fig. 3). The brilliant interference colors produced when a gypsum plate Red I is inserted in the field may be used to detect the location of cutinized lamellae and to indicate the orientation of the submicroscopic units of the wall. When fiber cell-walls are stained with chlorzinc iodide, congo red, Magdala red or certain other dyes they show dichroic phenomena, *i.e.*, they change color when rotated between crossed Nicols. This change in the intensity of color is due to the regular arrangement of the submicroscopic units of the wall (19). Cellulose walls are strongly dichroic, but lignified, cutinized or suberized walls do not show the phenomenon. This offers a method of detecting the early stages of lignification or cutinization in cellulose walls where it is not readily detected in other ways. Polarized light, therefore, permits the exact localization of isotropic lamellae such as the middle lamella in the wall, indicates the presence of crystalline units of submicroscopic size in cellulose, permits the determination of the orientation of these submicroscopic units and finally provides an invaluable method of detecting the lignification and cutinization of the cellulose walls.

5. *Fluorescent Light.* Fluorescence is the phenomenon by which the wave lengths of incident light are reradiated from objects in different wave lengths. The fluorescent light is usually of longer wave length than the incident light. If sections of lignified tissue are radiated with ultraviolet waves which are, of course, invisible to the eye, the tissue will become visible through the fluorescent light. The intensity of the fluorescent light varies with the degree of lignification so the progress of lignification may be followed by observing the tissue, mounted upon a quartz slide, with an ordinary microscope. Künemund (32) states that cambium cells do not fluoresce when wet but that the wood cells show definite fluorescence that increases with age. He was also able to

detect the presence of certain membrane constituents by the different color of their fluorescent light.

6. *X-ray Analysis.* The reflection of X-rays from the regularly arranged molecular groups in the cellulose wall produces spots or lines upon a photographic film placed in a position to intercept the reflected rays. From this spot pattern it is possible to determine the spatial relations of the molecular groupings (48, 49, 50, 51, 52, 53). This method of investigation has been applied to a study of plant cell-wall by numerous investigators and has made possible a determination of the actual size of the molecular aggregates in the wall, their distance from each other and their spatial relationships. It is further evidence that the plant wall is crystalline in nature.

7. *Ash Analysis.* If thin sections of tissue are carefully burned to remove all organic matter the ash will frequently remain in a distinct pattern. The residue of a wall may then be tested microchemically and the minerals present determined. This is of importance in that it is frequently impossible to detect the presence of mineral elements in the intact wall. Czaja (10) has used this method to show that intense staining of walls with ruthenium red may not be associated with the presence of pectic materials as has been generally assumed but is due to the presence of salts of calcium and phosphorous. These substances cannot be detected in the normal cell-wall but ashing the sections reveals their presence.

8. *Spierer Lens.* The Spierer lens differs from the usual oil-immersion objective by having a small mirror of aluminum, gold or silver deposited upon the vertex of the front lens. This mirror reflects some of the light entering the lens from the condensor. If the light from the condensor is stopped down until the beam entering the objective is totally reflected by the mirror, a dark field is produced. The light is reflected from the mirror to the object under examination and is again reflected from the surface of the object and enters the objective through the portion of the lens that is not covered by the mirror. The image produced by this lens consists of numerous alternating light and dark striations. In some instances the striations are broken up into short rods or irregularly shaped objects. This image has been variously interpreted. Seifriz (43, 45), who called attention to the lens and to

the unusual images that it produced, suggested that the Spierer image indicated the presence of definite structural units of ultra-microscopical dimensions. He contended that the Spierer lens provided further evidence for the micellar structure of the cellulose cell-wall. Thiessen (54) used the Spierer lens in studying the decomposition of the cell-walls of woods in relation to the structure of lignite and coal. The striations were assumed to be evidence of the existence of definite micelles. The essential similarity in the images produced by sections of sound and rotted wood cell-walls was considered by Thiessen as sufficient evidence for the existence of some close relationship between cellulose and lignin. The similarities in the Spierer images of thin sections of coal and those of cellulose membrane was considered to be evidence of the persistence in coal of the original cell-wall structure of the plants from which the coal was formed. These conclusions as well as those of Seifrizz must be critically regarded in consequence of the recent investigations of Clifford and Cameron (8). These investigators show that the Spierer image is probably the result of diffraction phenomena. Certainly it is no longer possible to accept measurements based upon Spierer images as indicating structural units characteristic of the material under observation, since similar images were obtained from such diverse substances as cotton fibers, asbestos fibers, salicylic acid crystals, iron wire and even from the lines on a stage micrometer (Plate Figs. 7 and 8).

Collenchyma cell-walls are known to consist of alternate lamellae of cellulose and pectic compounds. Either of these substances can be removed from the wall leaving the other remaining. Clifford and Cameron examined untreated collenchyma walls with the Spierer lens and then compared with this image the images obtained from collenchyma cell-walls from which cellulose or pectic materials had been removed. The same Spierer image was obtained in each case; no relation between the Spierer image and the structure of the wall could be detected. Berkley (6), in his recent study of the structure of wood, used the Spierer lens to bring out the gross structure of the wall but reported that he was unable to confirm the observations of Seifrizz and Thiessen upon the presence of diffraction patterns of micellar aggregates in Spierer images. It seems highly probable, therefore, that the

Spierer image has little or no significance so far as indicating the presence of fundamental structural units.

9. *Measurements of Refractive Indices.* Much may be learned about the structure of the wall from the refractive indices of the various lamellae. By mounting sections of tissue in liquids of varying refractive index the prominence of certain portions of the wall is greatly emphasized while that of other wall areas is diminished. This indicates the presence and gives the localization of different membrane materials.

10. *Hydration.* By the use of suitable reagents the wall of the cell may be greatly swollen. In the swollen condition, structural relations appear that are invisible in the unswollen state (Plate Figs. 4, 5, 6). Ball (5) has been able to count the layers present in a cotton fiber by using this method and to show some correlation between the number of layers and the number of day and night periods during which secondary thickening occurred. Sakostschikoff and Korsheniousky (41) were able to use this method to make counts of the layers in the cotton fiber but were unable to confirm Ball's theory of the origin of the layers since they found no consistent relation between the age of the fiber and the number of lamellae.

This brief description of the various methods employed by cell-wall investigators furnishes some idea of the way in which the data, upon which modern ideas of wall structure are based, have been obtained.

### *The Fine Structure of the Cell-Wall*

Three different conceptions of the fine details of cell-wall structure are current at the present time. The three ideas have certain features in common but differ in regard to the supposed size of the structural units of the wall.

The most widely held idea of the structure of the cellulose cell-wall does not differ essentially from the original Nägeli hypothesis. The important investigations of Frey-Wyssling (17, 20) with polarized light and those of Meyer (37), Meyer and Mark (38), Herzog (26), Clark (7) and others with the X-ray, indicate that the wall is composed of submicroscopic elongated units or micelles of crystalline nature. These crystalline micelles are separated from each other by a hydrophilic colloidal material. Figure 2B



from Seifriz (43) illustrates well the supposed arrangement of these micellar units. The crystalline character of the micelles is indicated by their double refraction between crossed Nicols.

Double refraction may be caused by the regular orientation of small isotropic rods imbedded in some medium. When the refractive index of the rods is equal to that of the surrounding medium, the system of rods and medium becomes isotropic. The cellulose wall of plant cells is doubly refractive when saturated with a series of liquids of varying refractive indices thus indicating that the double refraction is due to the crystalline nature of the micelles themselves and not to their orientation in the wall. This is confirmed by the results of X-ray studies for they also offer evidence of the existence in the wall of submicroscopic crystalline units. The presence of an intermicellar colloidal substance capable of swelling is evidenced by the swelling of the wall in water. In the swollen condition X-ray pictures show that the micelles themselves do not change in size but that distance between the micelles has increased. If the swelling of the wall were due, as Nägeli supposed, to the formation of adsorbed films of other films of water around the micelles, liquids such as ether and chloroform should likewise form films around the surfaces of the micelles and should cause some swelling. The failure of the cell-wall to swell when immersed in such organic liquids supports the idea that swelling is not due to the formation of liquid films around the micelles but to the presence of some intermicellar material of hydrophilic character. X-ray investigations also indicate the presence of some intermicellar material (26).

The internal structure of the micelle is also revealed by X-ray analysis. The basic cellulose group appears to be a long chain of glucose residues (glucose minus one molecule of water). A portion of one such chain consisting of two glucose residues is illustrated in Fig. 2A. The length of the chain is variously estimated by different investigators. Meyer (37) considers the chain to be composed of from 50-80 glucose residues. Freudenberg (16) estimates that the chains contain from 100-200 glucose units while Sponsler (48, 49, 50) considers the chains to be of indefinite length containing hundreds or even thousands of glucose units. The chain-like cellulose "molecules" are grouped into bundles in which they are arranged in parallel rows. These bundles of parallel



chains of glucose residues (cellulose "molecules") form the micelles. The number of parallel chains present in a single micelle is estimated by Meyer (37) to be from 40-60, by Freudenberg (16) to be about fifty. The micelle is the smallest morphological unit of natural cellulose. Meyer assigns a length of 15-30  $\text{m}\mu^3$  and a diameter of 2-5  $\text{m}\mu$  to these micellar units. Herzog estimates the micelles of hemp fiber to be 11.7  $\text{m}\mu$  long and 6.6  $\text{m}\mu$  in diameter, while Freudenberg considers the units in natural cellulose to have a size range of 50-100  $\text{m}\mu$  in length and a diameter range of about 6  $\text{m}\mu$ .

It is probable that not all of the micelles are of identical size in the same wall and probably the chains of glucose residues vary in size. So it is not possible to speak of cellulose molecules in the strict sense of the word. It is impossible that the celluloses in different species or even in different walls of the same individual organism are identical in their structural organization.

It is interesting to note that solutions of cellulose contain micelles of essentially the same size as those in the solid cell-wall (37). Various mechanical or chemical treatments may alter the size of the micellar units. Artificial fibers formed from solutions of cellulose show micelles of smaller size than those in the original native raw material (7). It seems probable that one reason for the lower tensile strengths of such artificial fibers is this decrease in the size of the micellar units.

The forces that hold the glucose residues together in long chains are the primary valence forces while the forces holding the chains together in the micelle are of the same order as those forces holding together the molecular aggregates of organic crystals—the forces of molecular cohesion. These can be measured in calories and Meyer (37) estimates that the forces in the case of a chain of fifty glucose residues amounts to about 1,200,000 calories. These tremendous forces, considerably greater than those holding the atoms together within the molecule, bind the chains of glucose residues together and are effective at the surface of the micelles themselves, there serving to bind the micelles together.

The micelles composing the wall are grouped into long fibrils in the case of fiber cells and these fibrillar units may be parallel

<sup>3</sup>  $\text{m}\mu$  = one one-thousandth of a micron; micron = one one-thousandth of a millimeter.

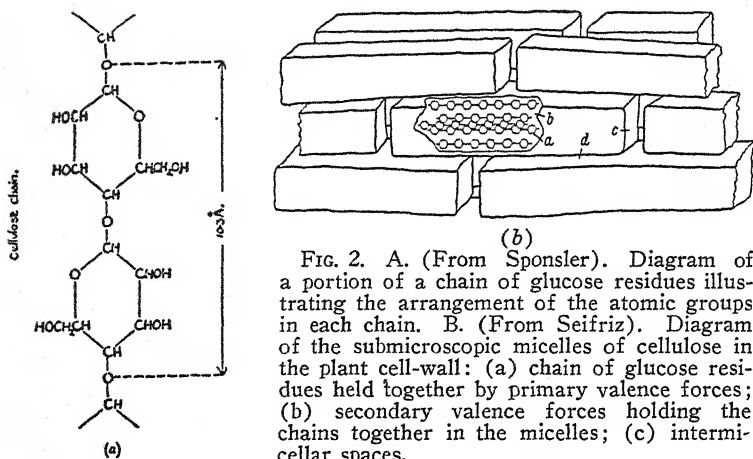


FIG. 2. A. (From Sponsler). Diagram of a portion of a chain of glucose residues illustrating the arrangement of the atomic groups in each chain. B. (From Seifriz). Diagram of the submicroscopic micelles of cellulose in the plant cell-wall: (a) chain of glucose residues held together by primary valence forces; (b) secondary valence forces holding the chains together in the micelles; (c) intermicellar spaces.

or inclined to the long axis of the fiber cell (Plate Figs. 5, 6). The angle of their inclination varies in different layers in the wall and may reverse its direction in the same layer of the wall as in the case of cotton (20). In some fiber cells the direction of the spiral fibrils is reversed in successive layers in the wall giving an alternation of right- and left-handed spirals in successive lamellae. Clark (7) reports that the angle of these spiral fibrils differs in compression wood from that in wood not subjected to compression. Jaccard and Frey (28) report a similar difference in the micellar orientation of wood under tension and that under compression. The latter investigators point out the rather surprising fact that this effect of tension and pressure was limited to the woody fibers and was not observed in the water conducting elements. The arrangement of the micelles in the bordered pits of coniferous wood has been reported by Frey (18, 20) as being that of concentric circles. This has been confirmed by the X-ray observations of Ritter (40). Frey reports a wide range of micellar orientation in the walls of different tissues (20).

Evidence of a different kind supporting the micellar hypothesis of cell-wall structure is presented by the studies of Gurewitsch (21) upon the permeability of the seed coats of certain seeds. The changes in the permeability of the cell-wall were explained in terms of alterations in the orientation of the micellar units in the wall especially as regards the size of the intermicellar spaces. The

presence of a hydrophilic intermicellar material is also indicated by the change in permeability produced by different reagents.

Czaja (10, 11), in extensive studies of metachromatic staining, finds that the micellar hypothesis adequately accounts for the differential staining obtained. K nemund (37), using metachromatic staining as evidence, describes variations in the size of the micellar units and differences in the size of the intermicellar spaces during lignification.

The second conception of cellulose cell-wall structure is that of Sponsler (48, 53) who was one of the first to apply X-rays to an analysis of fiber cell-walls. He differs from the other X-ray

workers cited in that he considers the X-ray evidence in support of the existence of micelles as definite entities in the wall, to be inadequate. Refraction patterns giving the impressions of micellar units may be the result of bending or warping of the material under examination. To quote (48): "It seems, then, that the very minute warped and bent regions in organized structures might readily be mistaken for individual blocks and measured by X-ray methods for micelles." Sponsler considers the wall to be composed of long parallel chains of glucose residues running lengthwise of the fiber. The chains are oriented in such a way as to form a space lattice of three dimensions. Figure 3C shows the arrangement of the glucose residue units in the space lattice. This arrangement forms a unit group that is  $6.10 \times$

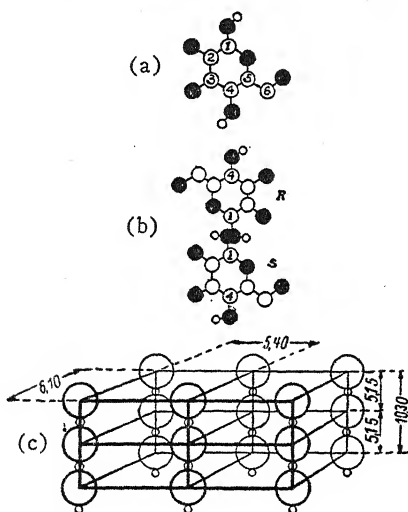


FIG. 3. (From Sponsler). (A). Diagram of the orientation of the atomic groups in a single glucose residue. Numbered circles represent carbon atoms; solid circles represent oxygen atoms; only two hydrogen atoms are shown. (B). Two glucose molecules placed as they are oriented in the cellulose chain to show the position of the OH groups at the time of condensation. Hydrogen atoms are represented by the small circles. (C). Spatial arrangement of the chains of glucose residues. Three glucose residue units in six different chains are illustrated. The dimensions are given in Angstrom units.

$5.40 \times 10.30$  A.U. (An Angstrom Unit is  $0.1 \text{ m}\mu$ ). In this diagram two vertical layers of chains are shown at right angles spaced 6.10 A.U. apart. The chains of glucose residues are spaced 5.40 A.U. apart in each layer. At right angles to these vertical chains, that is, in the horizontal position, three more layers of chains are shown spaced 5.15 A.U. apart. In the cellulose walls of fiber cells three planes of glucose residues are present. In ramie fiber the planes across the fiber are about at right angles with the fiber axis but this angle varies in other walls.

From this pattern it will be seen that the cellulose wall is considered to be a complex system built up of very thin molecular layers each composed of long chains of glucose residues. The surface view of one such layer is shown in Fig. 4. Certain

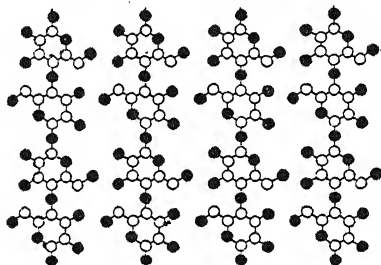


FIG. 4. (From Sponsler). Surface view of glucose residue chains forming one layer in the cellulose wall. The white circles represent carbon atoms, the black circles represent oxygen atoms.

layers extend lengthwise of the fiber wall, some extend across the wall and other layers form definite angles with the lengthwise layers. In the cylindrical fiber cell-wall the radial layers of chains are spaced 5.40 A.U., the tangential layers 6.10 A.U. and the vertical layers 5.15 A.U. from the center of one glucose residue to the center of the next adjacent residue. A single fiber cell-wall may be some 40,000 of these unit cell lattices in thickness. The wall of a fiber cell consists, then, according to Sponsler, of an enormous number of parallel molecular layers and not of a system of brick-like units as suggested by the micellar hypothesis.

Sponsler (50) suggests that since the inner surface of the wall adjacent to the protoplasm has a definite molecular pattern, the forces of crystallization at this surface may form a part of the mechanism by which glucose molecules become converted into cellulose (Fig. 3D).

The markings visible in cross-sections of fiber cells and upon the surface of the cell-wall bear no relation to the orientation of the glucose residue groups.

The third idea of cell-wall structure is that the wall is composed of definite cellulose units of visible size, each unit being enclosed in a thin film of non-cellulose material which serves to cement the units together. This concept has been supported by Lüttke (36),

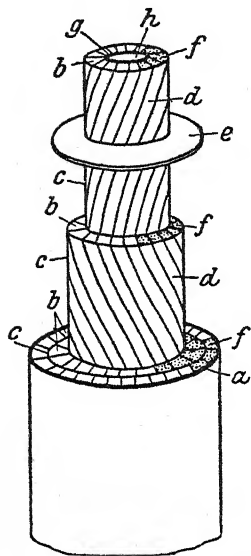


FIG. 5. (From Lüttke). Diagram of a small segment of a cellulose fiber cut away to illustrate the structure of the wall: (a) The primary cell wall. (b) Layers of the secondary wall. (c) Thin films of non-cellulose material separating the layers in the wall. (d) Spirally wound bundles of fibrils. (e) Cross element separating the fiber wall into segments. (f) Fibrils composing the spiral elements. (g) Innermost layer of the wall. (h) Cell lumen.

Hess (27) and more recently by Farr and Eckerson (12, 13). Figure 5 is taken from a recent paper of Lüttke (36) and illustrates his conception of cell-wall structure. The wall is built, according to this theory, in segments separated by definite cross elements. The lamellae so characteristic of thickened walls are believed to be caused by very thin films of non-cellulose material enclosing the cellulose itself. The vertical striations in the wall are similarly believed to be the result of thin films of non-cellulose material enclosing bundles of spiral fibrils. These thin films of non-cellulose material serve to act as a glue binding the cellulose segments together in the wall. The nature of the material composing these films apparently varies in different walls but the same structural system exists. The evidence for this structural system is based upon the behavior of cell-walls during swelling and solution in various reagents and upon the reaction of the fiber cells to certain dyes, especially chlorzinc iodide. This dye frequently fails to give the characteristic blue color when added to cellulose walls unless the wall is subjected to mechanical pressure. The application of this pressure, according to Lüttke, ruptures the thin non-cellulose film surrounding the cellulose and permits the staining reagents to reach the cellulose.

quently fails to give the characteristic blue color when added to cellulose walls unless the wall is subjected to mechanical pressure. The application of this pressure, according to Lüdtke, ruptures the thin non-cellulose film surrounding the cellulose and permits the straining reagents to reach the cellulose.

The recent papers of Farr and Eckerson (12, 13) seem to give additional support to this conception of cell-wall structure. They find that visible anisotropic crystalline particles can be demonstrated in the cell-wall and that by removal of "non-cellulose cementing materials" from cellulose particles with strong hydrochloric acid large numbers of cellulose particles can be obtained. They do not report the existence of any cross elements in the fiber wall, but their conception of a wall formed by the cementing together of definite cellulose particles with some non-cellulose substances suggests a membrane structure somewhat similar to the more elaborate plan advanced by Lüdtke.

Farr and Sisson (14), in a recent paper, suggest that the visible cellulose units reported by Farr and Eckerson may satisfactorily account for the X-ray patterns formerly thought to be the result of the existence of micellar units.

Hess (27) likewise describes the presence of "Blättchen" in the wall of cellulose fibers. He gives their size as ranging from 75-100  $\mu$  and suggests that these cellulose particles may be surrounded by thin films of non-cellulose material. He further points out that the X-ray diagrams of cellulose are better when the material is not thoroughly purified and freed from non-cellulose constituents indicating the orienting influence of the non-cellulose films. Hess is convinced that a system of films such as pictured by Lüdtke is an important structural feature of the fiber wall and bears a direct causal relationship to many of the properties of cellulose fibers.

#### *Modifications of Cellulose Walls*

Cellulose is not known to occur in plant cell-walls unaccompanied by other materials. The most widely studied modification of the cellulose wall is the change known as lignification that occurs in woody and other tissues. Lignin is an amorphous substance of unknown chemical composition that appears in many cell-walls that undergo secondary thickening. The first evidence of lignifica-

tion appears in the middle lamella where the pectic compounds are intensely lignified. Whether this change involves a partial transformation of the pectic material of the middle lamella into lignin or whether lignin is merely added to the pectic material already present, masking its appearance, is not definitely known. The careful work of Kerr and Bailey (29) has shown that both lignin and pectic material are present in the intensely lignified middle lamella of woody tissue. Kerr and Bailey show that Ritter's (40) conception of a middle lamella largely composed of lignin is based upon an objectionable method of removing lignin from the wall. Harlow (22, 25), in a series of papers, has also supported the idea that most of the lignin in wood is located in the middle lamella but further emphasizes that the presence of lignin need not exclude the presence of pectic materials. In a study of the lignification of wood cells Scarth *et al.* (42) found varying amounts of lignin in various portions of the wall with the greatest amount in the middle lamella. They were unable to demonstrate the presence of either cellulose or pectic materials in the middle lamella but were also unable to demonstrate the absence of these materials.

Lignin is present also in the secondary layers of the wall but Ritter (40), Harlow (22) and others have reported chemical and physical differences between the lignin of the middle lamella and that of the secondary layers of the wall. Lüdtke (36) considers that lignin is limited to the middle lamella and that the substance in the secondary wall generally classed as lignin is not lignin but a chemically different material that forms the thin films enclosing the cellulose constituents of the wall. The lignin of the secondary wall, if it is lignin, gives some but not all of the color reactions characteristic of the lignin of the middle lamella and differs in its resistance to solvents.

Lignification of cellulose walls does not alter the X-ray patterns of cellulose, so lignin does not involve a modification of the cellulose units themselves. In view of the dichroic effects of lignification (19) and the fact that either lignin or cellulose may be removed from a lignified wall without altering the structural relation of the other material, it seems probable that lignin is deposited between the cellulose micelles and involves a change in the intermicellar material rather than a change in the micelles themselves.



The process of lignification has been carefully studied in a variety of ways. Künemund (32), using metachromatic staining and fluorescent light, reports alterations in the micellar organization of the wall during lignification. The intermicellar spaces seem to vary in size. Kisser (30) also reports evidences of physical changes of the wall during lignification on the basis of the differential adsorption of certain dyes.

Freudenberg (16) considers two kinds of lignin: (a) primary lignin consisting of definite structural units formed in physiological synthesis and (b) secondary lignin built up from the chains of primary lignin to form large irregular three dimensional formations. The secondary lignin is considered to be a post-mortem condensation product of primary lignin. Hemicellulose is believed to be present with the lignin in the wall but neither compound is considered to penetrate the cellulose micelle, so most of the cellulose chains make no contact with the lignin in the wall.

Pectic compounds seem very commonly associated with cellulose in plant cell-walls. They may occur in definite lamellae in wart-like accumulations between cells (31) or may permeate the intermicellar spaces of the cellulose portions of the wall. Removal of cellulose with ammoniacal-copper-oxide from certain cell-walls leaves the pectic materials unaffected and in many cases the cell-wall appears unchanged in thickness, indicating that pectic materials have permeated the cellulose structure. Removal of the pectic material usually brings about a striking lamellation of the wall which suggests that definite lamellae composed largely or entirely of pectic material were present between the cellulose lamellae (1).

The striking affinity of pectic compounds for ruthenium red has made this dye a favorite test for the presence of pectic compounds. Kerr and Bailey (29), after a critical examination of this dye, find that while it is not specific for pectic materials it may be used with certain precautions as a strongly presumptive test for those compounds. Czaja (10) has reported evidence, however, to indicate that the adsorption of ruthenium red by tissues is no indication of the presence of pectic compounds but that it is determined by the presence of organic salts of calcium and phosphorous.

Pectic compounds make up the first cell-wall formed between the daughter nuclei, and subsequent layers of cellulose wall con-



tain more or less pectic material. As indicated earlier, pectic material is the first to be lignified and this has led to the suggestion that lignification is, in part at least, a change of pectic material in the wall.

Cutinized cell-walls have also been the subject of considerable study. Lee and Priestly (33) suggest that cutin reaches to the surface of epidermal cell-walls by migrating along the radial walls of subepidermal tissue. At the surface, exposure to light and oxygen bring about chemical and physical changes that produce the cuticle. Frey (18, 20) and Anderson (2) have shown that complicated relations between cutin, pectic materials and cellulose lamellae exist in certain epidermal walls. In some cases cutinized cellulose lamellae are deposited adjacent to the living protoplasm on the *inner* face of the outer epidermal wall (3). This suggests that cutin may be formed by the protoplasm of the epidermal cell itself and that cutinization of epidermal walls may not be directly related to exposure to dry air, light and oxygen.

Cell-walls are usually regarded by botanists as non-living structures produced by the living protoplast. There is evidence, however, that indicates a more active rôle for the cell-wall. Lloyd (34), in describing reproduction in a species of *Spirogyra*, observed that the bulging of the female gamete was not due to internal pressure but to changes in the wall itself. As a result of these observations Lloyd says, "Whatever the minutiae involved may be, it would seem that we are forced to recognize that there are qualities of the cell-wall in the living cell which may change—such change constituting a behavior of the cell as a living whole. In some measure we may assert the wall itself to be a living part of the cell." In another paper Lloyd and Ulehla (35) find that in the marine alga *Postelsia* some irreversible change similar to death occurs not only in the protoplast but also in the cell-wall.

Studies of the growth of cell-walls indicate that, in some cases at least, turgor pressure may not be the cause of the extension of the wall. Söding (47), in his study of cell-wall extensibility, finds that the first stages of cell-wall growth are not caused by turgor pressure. This point of view is disputed by other investigators. Cranmer (9) considered the wall as a living structure and believed the outer layers of the protoplasm were continuous with the intermicellar substance. While the possibility of vital

activity in the cell-wall cannot be denied, the evidence in support of this contention is inadequate at present to warrant its general acceptance.

The writer has attempted to call attention to recent developments and at the same time to convey some picture of the cell-wall as it appears to workers in the field. Many problems of cell-wall structure are still the subject of active discussion and debate and further discoveries may bring changes of greater or lesser magnitude to the ideas now current. A few generalizations may be safely made from facts so far accumulated:

1. The cell-wall is a laminated structure composed of many lamellae that differ in chemical and physical properties.
2. The skeletal framework of the walls of the higher plants is composed of cellulose.
3. Cellulose is composed of long parallel chains of glucose residues. These chains are definitely oriented in the wall but the exact method of their orientation is a matter of dispute.
4. The cellulose framework of the wall is permeated with non-cellulose materials of amorphous character.
5. It is possible that the plant cell-wall may possess some of the characteristics usually attributed to living material during certain phases of its growth.

The writer wishes to acknowledge his indebtedness to Professor Dr. J. Kisser of Vienna for calling his attention to certain papers of interest and importance.

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## EXPLANATION OF THE PLATE

FIG. 1. Outer epidermal wall of *Clivia nobilis* stained with Sudan III to show the distribution of cutin in the wall. Dark areas are cutinized cellulose, light area is composed of cellulose and pectic compounds (440 $\times$ ).

FIG. 2. Outer epidermal wall of *Clivia nobilis* saponified with hot KOH to remove cutin. Cellulose lamellae over each cell become visible and the lamellation of the innermost cutinized region becomes apparent. Cuticle resists saponification (440 $\times$ ).

FIG. 3. Outer epidermal wall of *Clivia nobilis* between crossed Nicols. The isotropic pectic materials in the wall appear as dark lines. The structural organization of the wall is revealed (500 $\times$ ).

FIG. 4. Small segment of a collenchyma cell-wall of *Lycopersicum esculentum*. Swollen and subjected to pressure. The cellulose lamellae composing the wall have been separated and flattened out (300 $\times$ ).

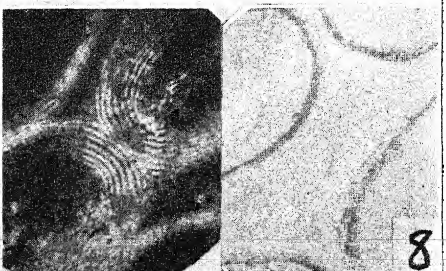
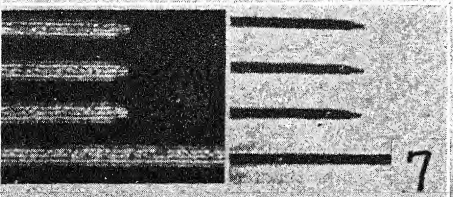
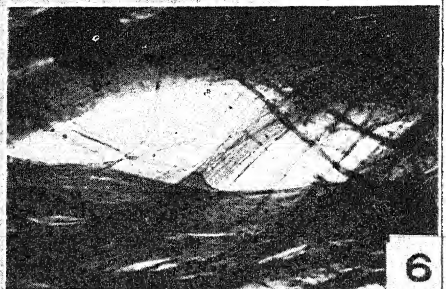
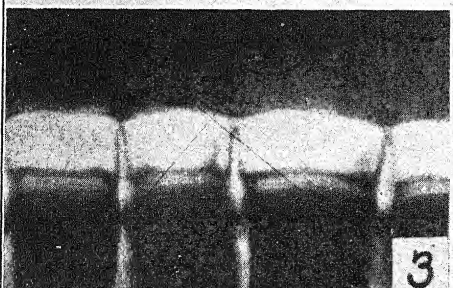
FIG. 5. Flax fiber swollen to reveal spiral fibrils composing the wall.

FIG. 6. Small segment of one flax fiber wall swollen and subjected to pressure (350 $\times$ ) to reveal its fibrillar structure. The swelling treatment has made the fibrils of the wall large enough to be seen (2000 $\times$ ).

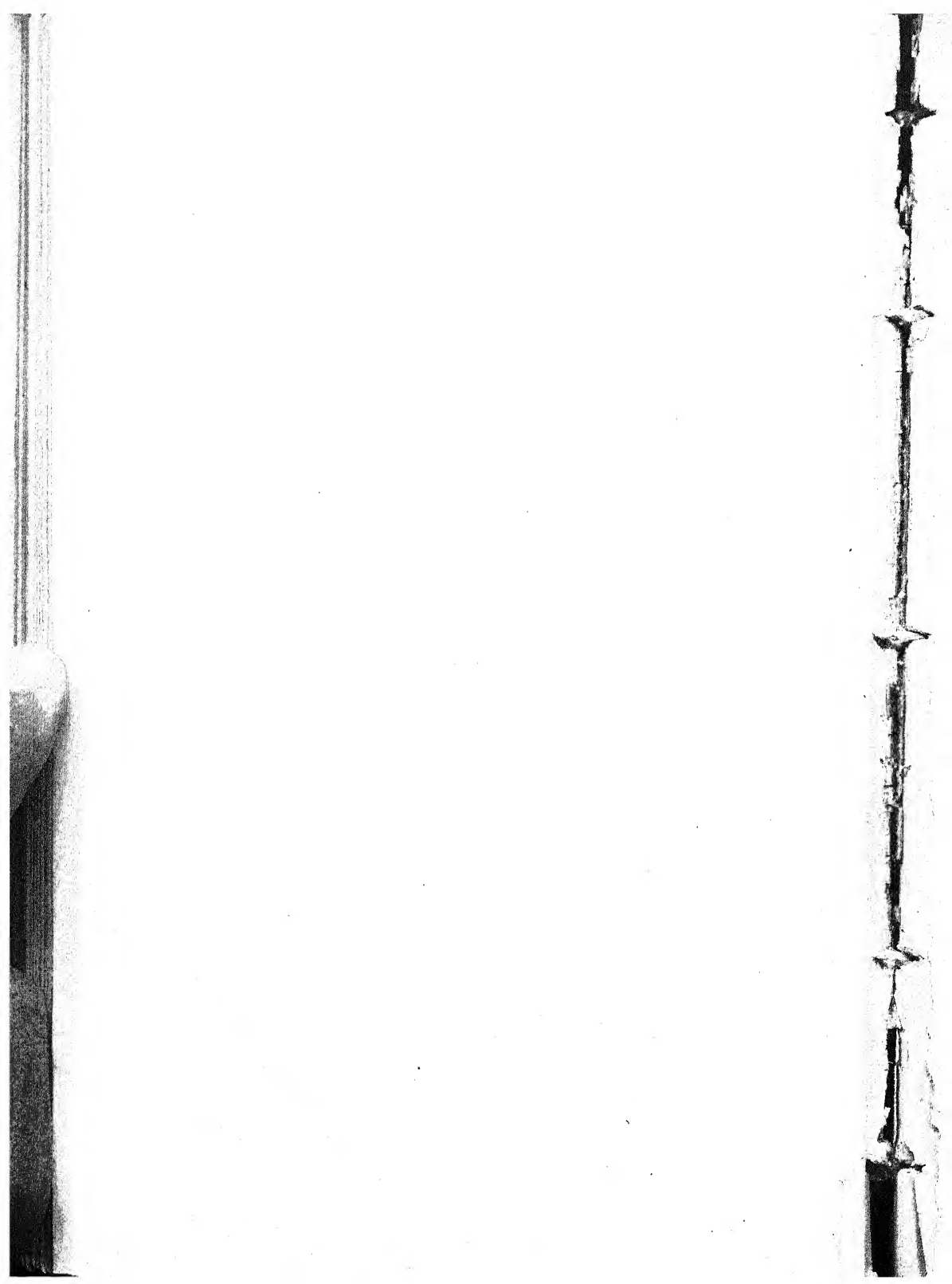
FIG. 7. (From Clifford & Cameron). A stage micrometer as seen through the ordinary oil-immersion microscope objective (950 $\times$ ) and as seen through the Spierer Lens (850 $\times$ ).

FIG. 8. (From Clifford & Cameron). Cross section of the collenchyma cell-walls of *Cyclamen persicum* as seen with ordinary oil-immersion objective (950 $\times$ ) and with the Spierer Lens (890 $\times$ ).









# THE BOTANICAL REVIEW

VOL. I

MARCH, 1935

NO. 3

## A REVIEW OF RESEARCHES CONCERNING FLORAL MORPHOLOGY

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In the following pages it is proposed to indicate the chief lines along which attempts have been made to solve the problems that have arisen in connection with the organisation of angiospermic reproductive structures. The treatment of the subject under three main headings is perhaps most likely to give a clear account of the work which has been done in the last few years: 1. the "old classical morphology"; 2. the "new morphology"; 3. "developmental morphology."

### 1. THE "OLD" MORPHOLOGY

It is a conventional and very generally accepted theory that the parts of a flower are the morphological equivalents of leaves (21); the implication is that they are transformed or metamorphosed leaves, an idea which is especially associated with the name of the German poet-philosopher, Goethe (28).<sup>1</sup>

As Asa Gray pointed out (29), the term "metamorphosis," as applied to the appendages of the aerial shoot of plants, is a *figurative* expression and is liable to mislead unless it is clearly understood that the "substance of the doctrine" is *unity of type*. That is to say, foliage leaves and floral organs may be of the same fundamental nature; but that does not necessarily imply that they are formed the one from the other.

The Goethean theory of metamorphosis is the underlying idea of the majority of the numerous researches into floral morphology from the time of its formulation until the present day. Two main kinds of evidence have been cited in its support, one from normally-developed flowers and the other from abnormal or teratological

<sup>1</sup> For accounts of the philosophical-botanical speculations of the latter part of the 18th and earlier part of the 19th centuries: 29, 54.

forms; and though the theory has become so deeply rooted and generally accepted that specific "proof" is no longer considered necessary, work is continued along these lines with two objects in view: first, that a more detailed knowledge of floral structure and organisation may be obtained; and secondly, that from this detailed knowledge a truly natural phylogenetic system of classification may ultimately be compiled (20).<sup>2</sup>

### 1. *Researches on normally-developed flowers*

For the moment, these may be considered as falling into two classes: (a) those which are concerned with the origin and development of the floral axis and of the various floral organs arising from it (organogenesis); (b) those which consider the structure, particularly the vascular anatomy, of the floral axis and its appendages (floral anatomy).

This classification is temporarily convenient since, in the past, such researches have been more or less independent.

#### (a) *Organogenetic studies*

The name chiefly associated with these is that of Payer (52). They have contributed to an understanding, not only of floral development, but also of comparative morphology and general floral plan as summed up, for example, in Eichler's "Blüthendiagramme" (24).

The original organogenetic observations were made directly on flower-buds from their earliest stages of development, observations involving great patience and extremely delicate manipulation. Modern researches on organogenesis have been facilitated by the use of the microtome; and while the older work was concerned simply with the composition of the flower and the origin and development of its parts, so far as these could be seen without sectioning, a new outlook is manifested in recent observations in that they are combined with other considerations, *e.g.*, cytological or anatomical.

Amongst morphological studies in which the development of the flower from the earliest stages is carried on through the cytological processes of the formation of pollen-grains and embryo-sacs to the

<sup>2</sup> It must be confessed that the newer researches do not seem to have greatly impressed the authors of the latest systematic works in which little or no mention is made of current ideas on the subject of Floral Morphology (53, 37; criticism of Rendle, 26).

production of embryo and endosperm, may be mentioned the work of Juliano and his collaborators in the Philippine Department of Agriculture (41-44) and that of Maheshwari and others at Agra, India (46, 47, 11). These careful researchers all add to the sum-total of detailed data which are of value in determining the relationships of the species dealt with.

(b) *Studies of Floral Anatomy*

The use of the microtome and the improvement of technique have made it possible to examine complete and uniformly orientated series of sections of floral structures in all stages of development from inception to maturity. In this way the form and order of appearance of floral appendages may be estimated and the origin and arrangement of their vascular system may be clearly apprehended. Not only must the structure be followed *from pedicel to apex of the flower* but observations of vasculature should be made from a *complete series of developmental stages in each case*; otherwise, deductions drawn from vascular anatomy may be misleading. That is to say, anatomical and organogenetic studies must be combined; and there is evidence in some of the later researches into Floral Morphology that this is being increasingly realised and that the old distinction between such studies—as, for example, in the work of Van Tieghem (98)—is being broken down.<sup>3</sup>

Anatomical studies of the flower date from the observations of Robert Brown who realised that more was needed than a knowledge of external form and of such features of venation as could be seen with the naked eye (6, p. 237). Brown's work was not followed up in England until Henslow, influenced by the work of Van Tieghem in France, published his observations on Floral Anatomy between the years 1876 and 1890 (34-36).<sup>4</sup>

<sup>3</sup> Arber (6) notes that since external shape and anatomical structure are two aspects of "form," *i.e.*, they are the products of differential growth and development of the same fundamental mass of tissue, form, anatomy and ontogeny should be studied as an "indissoluble whole." While advocating this method as preferable to the purely analytical work (without subsequent re-integration) of so many English investigators, Arber considers that Troll (99), in Germany, has gone too far in a holistic and synthetic treatment of the plant, his view being that form is a unity which cannot be analysed. As Arber points out, the unreserved acceptance of this attitude, to the exclusion of analysis, would bring scientific work to a full stop.

<sup>4</sup> An historical sketch of anatomical studies of the flower is given by Grélot (31).

A new impetus was given to research in Floral Morphology in 1922-23; it is impossible to give anything like a comprehensive review of the many detailed studies which have now been carried out and an outline of their general scope must suffice, supplemented by the bibliography.

*Carpel Polymorphism.* At the British Association meeting at Hull, in 1922, E. R. Saunders exhibited abnormal gynaecea of the garden Stock in which evidences of a 4-carpellary constitution were apparent.<sup>5</sup> Influenced by these examples, she made an investigation of normal Crucifers which led her to uphold, as typical for the family, the 4-carpellary theory suggested primarily by Lindley in 1828 (45) and adopted by various subsequent observers, in opposition to the conventionally-accepted 2-carpellary view as explained in the text-books.<sup>6</sup>

Basing her contentions on the venation of Crucifer carpels and on the lines of dehiscence of mature fruits, and axiomatically stating that each original vascular cord passing from the floral axis to the gynaeceum represents the supply of a whole carpel (67, p. 128), Saunders has advanced the opinion that carpels are not uniform in type but that in the course of floral evolution *two main forms* have appeared: "(a) the valve or hollow type which is more or less extended laterally and, if fertile, bears the ovules either singly or in a single row on either margin; (b) the consolidated type which, if fertile, produces ovules either singly or in from one to several rows on each flank of the midrib" (68). This latter form of carpel, it is claimed, may consist simply of a solid vasculated column, or of a radial plate of tissue, or it may show extensions on either side of the midrib, forming the semi-solid, or pseudo-valve, type. This semi-solid form is regarded as variable in the amount of extension it may display;<sup>7</sup> presumably, also, extensions need not necessarily be symmetrical on both sides of

<sup>5</sup> Reference will be made to these in the next sub-section.

<sup>6</sup> Historical sketches of the various views regarding the constitution of the Crucifer gynaeceum and the nature of its characteristic dissepiment will be found in (22, 25, 57, 93).

<sup>7</sup> Cf. the cases of the typical legume and that of *Haematoxylon*, both regarded by Saunders as *bi*-carpellary in constitution, the former consisting of a "cardinal" solid and sterile carpel, + a "vexillary" semi-solid and fertile carpel; and the latter of two equal semi-solid carpels, one fertile and the other sterile: 61, pp. 143-4.

the midrib.<sup>8</sup> Thus, while Saunders has retained the conception of the carpel as fundamentally a leaf-like organ, she regards it as capable of undergoing the modifications of form indicated above and has summed up her theory in the term "Carpel Polymorphism."

With regard to the typical members of the Cruciferae, Saunders' view is as follows (57): The gynaeceum of the silique type is composed of two lateral valve-like sterile carpels and two median consolidated carpels, each bearing ovules on both sides of its vascular cord; the dissepiment dividing the ovary into two loculi is regarded as true carpellary tissue, actual parts of the two solid carpels and *not* a "false septum" formed by a secondary ingrowth of tissue. The stigmatic function is performed by the tips of the two reduced fertile carpels in most types (*e.g.*, Wallflower)—the difficulty seen by Saunders in the acceptance of a "commissural" stigma is thus removed; or it tends to be confined to the two sterile valves, as in the Stock, though this condition is rare (57); or again in exceptional cases, both sets of carpels may form stigmas. The gynaeceum of the silicula type (*e.g.*, Shepherd's Purse) is held to be formed on similar lines, except that from observations of venation Saunders regards the two fertile median carpels as semi-solid and the valve carpels as considerably reduced in extent (61).

Having based her theory upon observations of the Cruciferae, Saunders proceeded to investigate a very wide range of Dicotyledonous and Monocotyledonous families (58 *et seq.*). She now claims to have found Carpel Polymorphism exhibited almost universally amongst the Angiosperms, even in cases where, originally, she accepted monomorphism.<sup>9</sup> She regards polymorphism as having arisen in many instances in connection with a considerable reduction in carpel number; this is the case, for example, in the *Ranunculaceae*, *Rosaceae* and *Phytolaccaceae*. In various members of these families, there is a large number of carpels, all of the

<sup>8</sup> Cf. McL. Thompson (93, pp. 8-10), on the number of carpellary types which may be envisaged according to Saunders' theory.

<sup>9</sup> See, for example, *Melanthium*, at first accepted as possessing a gynaeceum of 3 fertile valve carpels (61) but now claimed as having a polymorphic gynaeceum of 3 fertile and 3 sterile carpels (63); *Papaveraceae*, formerly regarded as containing certain forms (*Platystigma* and *Platystemon*) with valve carpels only (61), now held to include nothing but polymorphic types (70).

fertile valve type; but in those members where the carpel number is reduced, polymorphism is believed to occur (71).

Saunders claims that her theory of Carpel Polymorphism explains satisfactorily various points of floral structure which have in the past appeared as anomalies or presented difficulties of interpretation. Some of the points thus "explained" are: the nature of "false septa" and the constitution of "commissural stigmas";<sup>10</sup> the "so-called" terminal carpel; the cause of obdiplostemony; the origin of parietal and free-central placentation; and the gynobasic style. She has certainly rendered a considerable service to the cause of Floral Morphology by stimulating long overdue anatomical enquiry into these various points, for her revolutionary treatment of the subject has raised much comment and criticism. Approval of her theory is expressed in a review of the "Classification of Flowering Plants," in which Rendle is taken to task for not giving due consideration to Saunders' explanations of obdiplostemony and other points (26); and Dowding is convinced of Carpel Polymorphism in *Arceuthobium americanum* (Loranthaceae) and in the Santalaceae (19); but it is evident that the theory has not commended itself to the greater number of workers in the field of Floral Morphology during the last 8 years.<sup>11</sup> There seems to be a general impression that problems have been raised where none existed, as in the case of the terminal carpel (*v. infra*, footnote 13); and that in many instances complicated explanations of comparatively simple matters have been invented to fit in with the theory as, for example, in the case of *Arachis hypogea* which is claimed to have from 10 to 12 carpels instead of 1 because, according to the rules of Carpel Polymorphism, the 10 or 12 longitudinally-running vascular strands of the ovary wall must each represent the supply of an individual carpel (93).

A measure of support for Saunders' original observations has come from Eames and Wilson (22) and from J. Dickson (18),

<sup>10</sup> For an anatomical defence of the usual explanation of the commissural stigma as made up of the united halves of normally placed stigmas, and a criticism of its necessarily complicated derivation if the polymorphism theory is to be generally applied: 21, pp. 184-5; Text-fig. 29.

<sup>11</sup> For criticism and repudiations of the theory as a whole, or in part, see, for example, 3, 4, 5, 6, 9, 12, 13-15, 18, 21, 22, 23, 40, 51, 81, 93; see also summary and comments by Barton-Wright (10). Saunders' replies to certain of these criticisms will be found in 62, 67, 75 and 77, where she re-states and extends her claims.



in so far that in the Cruciferae and certain other Rhoeadalean forms, these workers agree that the gynaecium is composed of carpels *differentiated* as "solid and fertile" and "valve and sterile" or, as Dickson prefers to term them, "*contracted* and fertile" and "*expanded* and sterile."<sup>12</sup> Their support is the more cogent in that they have added data from the vascular supply of the carpels; they hold that the carpel is fundamentally a 3-trace structure and that the vascular supply to the "solid" carpels, although greatly concentrated, is of the same nature and constitution as that to the "valve" carpels. They differ from Saunders in that they do not believe in the existence of the "semi-solid" carpel, as conceived by her.

So far, Eames and Wilson on the one hand and Dickson on the other are in agreement. Eames and Wilson, however, regard the solid carpels of the Crucifer gynaecium as having arisen by the phylogenetic elimination of the loculus of *closed* carpels, fused with one another across the centre of the gynaecium, and the dissepiment, as it now appears, to be the attenuated result of the contraction of these two carpels which are protected and partly enclosed by the other two, *open*, carpels (23). Dickson, on the other hand, has traced a series of forms through the Rhoeadales which may be interpreted as indicating the possibility that the gynaecium was ancestrally composed of *open* carpels only, fused margin by margin; in certain types a *lateral* contraction of alternate members is apparent, as in *Glaucium* and *Sanguinaria*, accompanied by restriction of ovule-bearing to the contracted carpels; contraction reaches its extreme expression in such forms as *Macleaya* and *Chelidonium*. The original conception of the dissepiment as an outgrowth of carpellary tissue appears to be justified by the course of events in *Glaucium* where, *after* formation of the ovules, a spongy embedding tissue grows out from the contracted fertile carpels, forming a loose "septum" across the ovary. In the Cruciferae the septum is of very early formation and it is of more coherent tissue than in *Glaucium*; but there is no reason to believe that it is anything but an extension of carpellary tissue, entirely comparable with that in *Glaucium*. In the Capparidaceae and

<sup>12</sup> J. Dickson's work is at the moment unpublished; a preliminary communication on the Papaveraceae has, however, been made by her to the Linnean Society, and the complete paper will shortly appear in the Society's Journal.

Fumariaceae the septum is not formed at all while in *Sanguinaria* slight traces of outgrowing tissue may appear at the base of the ovary.

Although, according to Dickson, carpel-differentiation seems to be characteristic of the Rhoeadales as a whole, the more primitive members of the *Papaveraceae*, *Platystemon*, *Platystigma* and *Romneya*, at least, possess carpels of uniform, undifferentiated type, the gynaeceum of *Platystemon* showing, in construction, distinct similarities to that of certain *Ranunculaceae*.

Apart from the Rhoeadales, no example of carpel-differentiation has been found either by Eames and his collaborators or by Dickson though many families have now been investigated.

Special note should be made of the work of A. Arber in this connection. This author does not favour the theory of Carpel Polymorphism and, with regard to the interpretation of the Crucifer gynaeceum, she holds that the anatomical basis of the 4-carpellary view does not stand examination; she believes that to ask how many carpels enter into its composition is an unanswerable question, for in this family the gynaeceum "may never in the whole course of its history have passed through a stage in which it could be said to consist of distinguishable leaf members" (3). This latter statement is probably indisputable; but, as against the retention of the bi-carpellary theory "*as an instrument of description*," it may be suggested that the comparative work of Dickson on the *Papaveraceae* and other Rhoeadalean forms now indicates that the 4-carpellary theory would provide a better "instrument."

Since it does not appear that the theory of Carpel Polymorphism can be accepted as generally proven, it is not necessary here to examine categorically and in detail those points which it is held to explain; reference to a consideration of the main items from the view-points of Saunders and her opponents will be found in the works cited below.<sup>13</sup>

<sup>13</sup> Explanation of the "false septum" and "commissural stigmas" have already been noted in dealing with the Cruciferae. With regard to other points, the references are as follows:

(1) *The terminal carpel*. In the Leguminosae, for example, Saunders disposes of the "difficulty" by the theory of Carpel Polymorphism, according to which  $G=2$ , not 1, in this family (70, 63, 69, 71). Arber (2) and Eames (21) show how, by extreme reduction of apical tissue in determinate shoots, the terminal leaf-member, carpellary or otherwise, may become a reality.

(2) *Obdiplostemony*. Saunders has examined this in many families (61, 64, 73, 74, 75, 77) and concludes that it arises through Carpel Polymorphism.

*The Inferior Ovary.* As a result of her "Leaf-skin" studies and consideration of gynaecia, Saunders has expressed the view that the old term "epigynous," for those floral types with an "inferior" ovary, should be discarded in favour of "syngynous" (58, 59), or "syngonous," as it appears in later papers (63, 69, 74). The change of terminology is suggested on the grounds that the enclosing wall of the ovary is exclusively foliar, conclusions being drawn in the first place from certain Iridaceous forms and from *Begonia*. While it is true that in some of the Iridaceae and Amaryllidaceae (e.g., *Alstroemeria*) there are distinct evidences that the lower parts of all the whorls, perianth, androecium and gynaecium, are conerescent,<sup>14</sup> it does not seem possible that this

In the case of *Pelargonium*, Bancroft and Dickson have shown that the cause of obdiplostemony is the carrying-out of the petal-stamen bundles by the petal midribs, so that petals + petal-stamens act as a single whorl (9); in the course of alternation of whorls, it follows that the carpels are developed opposite the petals. The same observation has been made in other families by these writers and also, in fact, by Saunders herself (77); in this paper Saunders claims that the carrying-out of the petal-stamen bundles by the petals allows space for the antepetalous carpels to develop loculi, while the antesealous carpels remain solid, their development being hampered by the presence of the antesealous stamens. This view would, of course, be reasonable, if Carpel Polymorphism could be accepted as a fact; but even so, it is difficult to see how Carpel Polymorphism could be the cause of obdiplostemony.

(3) *Free-central placentation.* In the Primulaceae, for example, Saunders holds that the free-central placenta is composed of fertile solid carpels, while sterile carpels form the wall of the ovary (75). In an as yet unpublished paper, Dickson has been able to show the connection between such 5-carpellary types as *Aquilegia* (Ranunculaceae), Geraniaceae, certain Silenoideae and the Primulaceae, a series in which no kind of carpel differentiation has been detected. The free-central placenta of the Primulaceae seems to be composed of fused carpel-margins ( $\pm$  residual axial tissue), detached from the wall of the ovary as they are in the Silenoideae, where, however, the detachment takes place ontogenetically. Cases of *parietal placentation* are explained by Saunders as in the Cruciferae (74); but that recourse to Carpel Polymorphism as an explanation is not universally necessary is shown, for example, by the case of *Platystigma* (18); reference should also be made to Bugnon's refutation of Carpel Polymorphism in *Drosera* (15).

(4) *The gynobasic style.* Under the Carpel Polymorphism theory, the gynobasic style is derived in various ways (59, 63). Work is being carried out at the Imperial Forestry Institute (Oxford) which indicates simpler derivations on the "monomorphic" view; this point must, however, be left until the evidence is complete.

<sup>14</sup> In a paper read before the British Association at Leicester in 1933 (38), Hyde produced evidence of "syngony" in the Campanuloideae. It may be noted that this paper is of interest in that Carpel Polymorphism and the conventional De Candolle view of the carpel are alike refuted in favour of yet another suggestion, namely, that the gynaecium of the Campanuloideae is composed of one whorl of similar carpels, each enclosing a loculus and a bifid "ovulphore," which arises ontogenetically from the floral axis and

is necessarily always the case. Bugnon has definitely repudiated the idea for *Begonia* where he holds that the wall of the ovary is *wholly axial*, from base to rim, and throughout its thickness (16). Moreover, instances of "floral cups" are demonstrable in certain perigynous Rosaceae which are axial in the lower part and foliar above (12, 39); and the possibility, in other perigynous forms, of cups which are axial from base to rim cannot be overlooked. From these latter types, the derivation of genuinely epigynous examples in which carpellary tissue is conrescent with the linear surface of the floral cup, is not unthinkable.

## 2. Researches on abnormally-developed flowers

Opinion is divided as to the value of abnormally-developed flowers in morphological interpretations. On the one hand are botanists who treat them as valueless;<sup>15</sup> on the other hand, there are many investigators who regard them as reversionary and, therefore, as "genealogical signposts," as Arber has termed them, to ancestral structures, or as indicators of morphological status.<sup>16</sup> A. P. De Candolle gave an impetus to the study of abnormalities from this point of view (61, 62) and he has had a considerable following (48, 102). Saunders, as already noted, was led to her conclusions concerning "polymorphism" of carpels in the Cruciferae by observation of teratological examples. Four-valved Stock fruits were found at the base of inflorescences where, she maintained, ontogeny might be expected to repeat phylogeny; these fruits were, therefore, claimed as reflecting an ancestral condition. Arber, however, points out that there is too great a tendency to choose, for discussion of this type, those abnormalities which fit in with preconceived ideas (4). She herself has found, at the base of inflorescences of *Nasturtium officinale*, flowers which have accessory flowers associated with each petal; to regard these as "ancestral" would be manifestly absurd. Further, as Arber notes,

carpellary axil. (The writer of this article is indebted to Mr. Hyde for notes concerning his work, which, it is hoped, will be published in due course.)

<sup>15</sup> See, e.g., the work of Gregoire who considers that the appearance of a leaf-like structure in the place of a stamen or carpel does not prove that stamens and carpels are modified leaves. He bases his objections to the conventional interpretation of such abnormalities on his observations that leaf-primordia and those of stamens and carpels are not identical (30).

<sup>16</sup> Cf. the work of Heinricher on proliferated *Primulas* which are held to demonstrate the origin of the ovules from leaf-tissue (32, 33).

most of the cases of so-called reversion which have been advanced against Dollo's "Law of Irreversibility in Evolution" (1), are meristic variations in which the number of parts is *higher* than normal; and there is no justification for the assumption that abnormalities of this kind are atavistic—they may equally well be futuristic.<sup>17</sup> Following Godron (27), she suggests that there may be a physiological interpretation of the abnormalities noted by Saunders and herself; for the lowermost (outermost) flowers of an inflorescence are free from the pressure which those towards the centre mutually exert upon one another; they are also more likely to be generously supplied with food-materials.

Arber, therefore, suggests a third attitude towards abnormally-developed flowers: scepticism with regard to their atavistic interpretation should be combined with recognition of their significance in the whole problem of causal morphology.

In connection with physiological interpretations of abnormally-developed flowers, reference may be made to the work of Schoute who regards meristic variation in general as "true variability," an expression of plasticity of the "flower-material," so to speak (79, 80).

A similar conclusion is reached by Müller, working especially on *Libertia*, an Iridaceous type in which none of the many variations is regarded as either reversionary or "futuristic" but as the result of environmental action on the plastic characters of the plant (49).

### 3. *Phylogenetic considerations*

Brief reference must be made under the heading of "The Old Morphology" to the very general belief that a relationship exists between the Angiosperms and the Cycadophyta (7, 8, 50, 100, 101). This belief is the outcome of a certain similarity of floral organisation in the Cycadeoids and the Angiosperms; and it has recently been considerably reinforced by the discovery of close resemblances in detail between the wood anatomy of the Cycadeoids and that of the Magnolias (101).

Comparisons have in the past been drawn between the microsporophylls of the Cycadeoids and the stamens of Angiosperms; and between the megasporophylls of *Cycas* and the carpels of

<sup>17</sup> Cf. also Worsdell, who believes that most cases of *simplification* are "progressive" rather than "reversionary" (102, Vol. 1, p. 5).

Angiosperms. The general conviction that these gymnospermic reproductive structures are obviously foliar has supported the accepted theory of the foliar nature of the angiospermic stamens and carpels.

The similarity of floral organisation noted above may, of course, be deceptive; and the comparisons between the spore-bearing organs of Angiosperms and Cycadophyta may be unwarranted. At the moment, however, it seems impossible to avoid the conclusion that some relationship, community of origin, at least, exists between the two groups. If, therefore, as H. H. Thomas suggests, the individual spore-bearing organs of the Cycadophyta are branch-systems rather than leaves, it seems that the classical morphology must be superseded in the case of the Angiosperms also, as explained in the following section.

## 2. THE "NEW" MORPHOLOGY

According to H. H. Thomas, the angiospermic flower is *not* the homologue of a vegetative bud (85); the clue to modern floral structures is to be found in the reproductive bodies of the Caytoniales, a Lower Jurassic group which was already angiospermic though manifestly of Pteridosperm affinities. The Caytoniales includes certain leaves and micro- and megasporangial structures which have not been found in organic connection but which, by association and from presumptive evidence provided by morphology and structure, are considered to belong to related forms (84). The leaves, *Sagenopteris*, may be regarded as generally angiospermous in type. The microsporangial structures, *Antholithus Arberi*, were pinnate in organisation, the apex and lateral branches showing dichotomous divisions, some of which were terminated by groups of "anthers," each with 4 longitudinal pollen-sacs. The megasporangial structures, *Gristhorpia* and *Caytonia*, were pinnate also; the branches were, however, not truly lateral, but arose from the upper surface of the axis; they consisted of shortly-stalked "ovaries" with basal, downwardly-directed "stigmas." There is no doubt that these "ovaries" were truly angiospermic, for each of them was a closed cupule, containing two rows of ovules produced along a line running from its stalk round its periphery; but whether the closed ovary of the Caytoniales bears any direct relation to the modern Angiosperm carpel is debatable.



In comparing the reproductive organs of the Caytoniales and of the Angiosperms, Thomas sets aside all preconceptions based upon the "old" morphology and proceeds to argue from new evidence, drawn from recent discoveries of primitive land-plants (Psilophytales), from researches on the Pteridophyta and from an increased knowledge of fossil Gymnosperms. This evidence, as Thomas notes, indicates that sterilisation of structures originally fertile is much more likely to have taken place than the metamorphosis of leaf-like structures into reproductive organs (84).

Starting, therefore, from *known forms* of fossil microsporangial branch-systems and postulating the operation of the processes of reduction and fusion, Thomas sees the product in the present-day *stamen* of the *Ranunculus* type, or in the *staminate flower* of *Populus* or *Juglans* (86);<sup>18</sup> and, from known forms of fossil megasporangial branches, he sees the same processes resulting in follicles of the *Caltha* type (84).

Some account of the suggested derivation of the follicle from the *Gristhorpia* type of megasporangial branch-system is necessary. From a study of venation, Thomas concludes that this apparently simple modern structure is fundamentally compound, consisting of an axial portion, and two ovule-bearing branches—such a structure, in fact, as might be produced by the reduction of a megasporangial branch of *Gristhorpia* to a short axis and a pair of ovaries (84). The dorsal vein of the follicle may represent that of the axis and the two marginal veins the ovule-bearing midribs of the ovaries. If the three segments of this reduced *Gristhorpia* became fused, a further reduction, involving the adjacent segments of the "ovaries," might take place, and the ovule-bearing midribs thus come to lie side by side. The stigmatic tissue, originally basal, extended upwards, reaching the apex of the now completely-evolved follicle (84).

Thomas seems to regard the penultimate tripartite structure with its three main veins (84) as a "palmate sporophyll"; this

<sup>18</sup> The derivation of the staminate flower of *Populus* is suggested from microsporangial structures such as those of the Upper Carboniferous *Crossothea* and the Triassic *Pteruchus*. According to this view, the reproductive structures of *Ranunculus* and *Populus* are not strictly comparable and the polyphyletic origin of the angiospermic flower is thus suggested. Against this suggestion of polyphyletic origin, of course, is the uniformity of endosperm-formation in the Angiosperm embryo-sac (Cf. remarks by Ramsbottom in 86, p. 42).



terminology, it should be noted, suggests a measure of convergence between the new and the old morphologies, for Eames considers the carpel of Angiosperms to have been derived from a 3-lobed, 3-veined, palmate *leaf* (21). Under the circumstances, Thomas's term tends to convey an inaccurate impression of his derivation of the follicle. If, however, according to Arber's conclusions, axis and leaf are not to be treated as "discrete morphological entities" (2) distinctions between branch-systems, leaf-like branches and leaves finally disappear under the operation of "reduction and fusion" processes; and the differences between the derivation of the carpel from a "branch-system" and from a "leaf" are more superficial than fundamental. But the question really resolves itself into one of the *ultimate origin* of lateral appendages of the axis: they may be cladode megaphylls (modified branches) or microphylls, arising from the axis by enation. Bower's very cogent suggestion that the flower should be regarded as a strobilus bearing appendages very probably of *both* types, must be given due consideration; and against the *literal* interpretation of the theory of metamorphosis—which is still, unfortunately, only too prevailing<sup>19</sup>—must be set his dictum that "normally produced sporangia antedated any other constituent part of the flower upon which those sporangia are now borne" (86).

### 3. "DEVELOPMENTAL" MORPHOLOGY

An entirely new outlook on Floral Morphology is suggested by McL. Thompson (87-97) who, holding the opinion that the subject cannot be further advanced by an intensive study of mature structures (either recent or fossil), proposes instead a study of "developmental" morphology, in which it is held, as a fundamental principle, that number of floral parts, fertility, sterility and the development of the stigmatic function are matters of growth-physiology and of the chemistry of cell-nutrition (55).<sup>20</sup> Thompson believes that if angiospermic floral types are studied ontogenetically from their earliest stages to maturity, they may be their own interpreters, without recourse to any phylogenetic considera-

<sup>19</sup> The idea of metamorphosis, it should be noted, was confused from the outset; Goethe, himself, used the term sometimes quite literally and at others in an idealistic sense.

<sup>20</sup> Developmental studies, based upon the same principles, are being pursued by Stirling in an endeavour to elucidate the phenomenon of heterostyly (82, 83).

tion other than the assumption of phyletic diminution of sporogenous tissue with accompanying reduction of apical growth (97).<sup>21</sup> He has, accordingly, made developmental studies of the Leguminosae (88-90, 93, 94), the Lecythidaceae (91) and the Scitamineae (95); and from evidences of advancing sterility in these families he has been led to a new theory of "the state of flowering known as angiospermy" (96, 97) in which he visualises the basis of the modern flower in a "sporogenous axis, devoid of obligate appendages."

Briefly, the evolution of the floral organs from this axis is suggested as follows: The lower part of the axis is sterile and ultimately produces sterile appendages, bracts, bracteoles and sepals. The base of its sporogenous tip is potentially microsporangial; from it emergences arise which ultimately become stamens; sterilisation of the lowermost microsporangial emergences results in petals or staminodes. The final positions of bracteoles, sepals, petals, staminodes and stamens are determined according to the maintenance of apical growth, or its replacement by toral growth. When toral growth becomes dominant over apical growth at an early stage and extends beneath the bracteoles and sepals, the maturing axis is cup-shaped and the "condition of the flower with inferior or semi-inferior ovary is initiated and is later attained if the dominance of toral growth is continued." The definition of "microsporangium" is closed by the production of emergences which are stigmatic and form "stylar components." The remaining axial surface which lines the cup is megasporangial; emergences from it ultimately become ovules. ✕

Thus, in the case of types with semi-inferior or inferior ovaries, Thompson disposes of the conception of carpels entirely: the Scitamineae, for example, are described as "acarpous" (95).

With regard to types with superior ovaries, he holds that the only essential difference from those with inferior ovaries lies in the fact that, in their case, apical growth is maintained until the general form of the floral axis is determined. The whole surface tissue of the apex of the axis is potentially sporogenous and, after the cessation of stamen-production, there arise from it emergences

<sup>21</sup> The writer is greatly indebted to Professor McLean Thompson for permitting her to read his conclusion in MS. before the actual publication of his work.

which, by processes of initial enlargement by apical growth, local sterilisation and union, result in "ovuliferous phylloclades" (*not carpels*, according to the general conception); ovule-production becomes restricted, the placentae are thus defined and the ovules finally become enclosed, such gynaeceal types as the legume and the follicle being produced (93, 94, 97). In these cases, Thompson holds that the emergences which eventually produce the "ovuliferous phylloclades" are potential stamens; their union marks the cessation of their initial apical growth; their development of stigmatisation is associated with their diversion from microspore-bearing and the initiation of the megasporangial phase. Thus, it appears to Thompson that there is an interplay and interdependence between the androecium and the gynaeceum which cannot, therefore, "profitably be discussed apart in matters of development or descent." The conclusions which result from this view of the evolution of the angiospermic flower are that "a species with few carpels *per* flower has not descended from ancient forms with many carpels, some of which have been suppressed"; and "neither is the flower with superior ovary, as now known, considered primitive, nor is the flower with inferior ovary considered advanced. They express distinct states of flowering which may exist simultaneously in a single accepted affinity" (97).

It is interesting that his studies of developmental morphology have led Thompson to a theory of the strobiloid origin of the angiospermic flower which has certain resemblances to that suggested by Bower (*v. supra*) though it presents a simpler view of the origin of the various floral parts, in that, according to Thompson, they are all derived by "emergence" from the axis.

Attention should be drawn to certain of Clapham's comments on Thompson's work (17); this author notes that while it is "not difficult to agree that the carpel, growing for the most part by intercalary elongation *after* infolding, is no longer a leaf whatever its form in ancestral types," "there seems to be no strong reason, on grounds of structure and development, for calling it phylloclade rather than leaf." There truly is need, as Clapham says, for a revised morphological terminology—or at any rate, for a more meticulous use of existing terms.

Further, Clapham does not consider that the "acarpous" explanation of the Scitamian gynaeceum is legitimate: "the inferior

ovary is surely a derivative type in which the ancestral carpels have ceased to bear ovules and are represented only by the "stylar components"; and while, according to Clapham, the Scitaminean ovary is not now composed of carpels, "it cannot be maintained that it is ancestrally acarpous." The present writer would agree that all stages between epigyny and hypogyny are known and that the differences of condition seem to be determined by the distribution of growth after initiation of the carpels on the receptacle (16); but she does not hold it necessary to regard epigyny as having arisen in only one way (*v. supra*). Thompson's view of the existence of superior and inferior ovaries within single cycles of affinity is accepted as incontrovertible (*cf., e.g.,* Hutchinson's new classification of the Liliaceae and Amaryllidaceae (37); but it is difficult to imagine that there is no serial and evolutionary connection between the two conditions.

It is regrettable that Thompson's studies of developmental morphology should not have included detailed observations of vascular anatomy; for while it is true, as he has said, that vascular anatomy is apt to be variable even within a species, experience (of the Primulaceae, for example) has indicated that if a sufficient amount of material is examined and if a sufficient number of developmental stages are followed serially, a fundamental plan of vasculature will be discovered which it does not seem possible to neglect in morphological determinations. As Arber admits, the *general scheme*, at least, of the floral vascular system "may have some value as indicating the broader trends of race history" (6); if that is so, while caution is undoubtedly necessary in interpreting floral anatomy, it is not desirable to set it aside entirely. Rather, it should be treated from the physiological standpoint Thompson himself advocates in dealing with developmental morphology as a whole and which Grélot, as long ago as 1897, realized as imperative (31). As he pointed out, in considering the structure of the bicarpellary Gamopetalae, floral parts show a great plasticity according to physiological needs and modification of the vascular tissue corresponds to modification of the ground tissue, the more so as the centre of the flower is approached. It is failure to realise the physiological aspect of vasculature, as Arber remarks (6) which has led Saunders to misinterpret the more strongly-developed vascular cords of the gynaeceum as the midribs of dis-

tinct carpels, for she has not considered the modification in venation of the carpels (as compared with foliage leaves) which has accompanied the shifting of function from midrib to margins.

In conclusion, then, it is evident that not only must considerations of angiospermic flower form and structure, both normal and teratological, be combined with developmental studies and checked by reference to palaeontological facts and to the reproductive structures of other living groups; they must be approached in relation to the problems of organic form and function as a whole; no line of investigation must be overlooked. The problems of organic form and function, however, as Arber remarks, await not only solution but even their full formulation.

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December, 1934.

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## GLOSSARY

*Enation*: outgrowth from the surface of an organ.

*Epigyny*: apparent attachment of sepals, petals or stamens to top of ovary; regarded as advanced stage.

*Free central placentation*: occurrence of ovules on a central axis in a one-loculed ovary, axis being unattached at its upper end.

*G=2*: numeral indicates number of carpels composing gynoecium.

*Gynobasic*: arising from side or base of ovary.

*Hypogyny*: attachment of sepals, petals or stamens beneath ovary; more primitive than epigyny.

*Inferior ovary*: ovary of epigynous flower.

*Meristic*: pertaining to or divided into segments.

*Obdiplostemony*: condition wherein outer stamens stand opposite petals.

*Parietal placentation*: occurrence of ovules on peripheral wall of ovary.

## THE CYTOLOGICAL ANALYSIS OF SPECIES-HYBRIDS\*

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The cytological analyses of hybrids during the past decade have been of great value in determining the probable relationships and origin of many species of plants. The combination of cytological and genetic investigations has been so successful, especially in the analysis of cultivated plants, that the union between these sciences has been recognized as a permanent hybrid—"cytogenetics." A union between cytology and taxonomy also has been effected during recent years, but "cytotaxonomy" is not so well established.

A hybrid may be defined as an individual resulting from the union of unlike gametes. The gametic differences may involve gene mutation, chromosome morphology, chromosome number or various combinations of these variations. The parents may differ only in gene constitution in most varietal hybrids and apparently in certain species-hybrids. Most of these hybrids are fertile but genetic factors may cause meiotic irregularities and gametic sterility. Most hybrids derived from parents differing in chromosome structure or chromosome numbers are at least partially sterile.

### *Factors Involved in Chromosome Pairing*

In most intraspecific hybrids, the chromosomes behave as they do in the parental forms. The genetic evidence shows that there is a gene by gene pairing of homologous chromosomes at the prophase of the meiotic division. The cytological picture also shows, in favorable material, the pairing of similar chromomeres in the homologous chromosomes. The homologous chromosomes are usually held together until metaphase by one or several chiasmata, *i.e.*, an exchange of partners between paired threads or chromatids, of the bivalent chromosome. Although the chiasma frequency may be controlled by genetic and environmental factors, it can be used with caution as a rough index of chromosome homology. In most intraspecific hybrids, the meiotic divisions are regular and less than ten per cent of the pollen is aborted.

\* A glossary appears at the end of the article.

It has been assumed that the degree of chromosome pairing in species- or generic hybrids can be used as an index of chromosome homology and species relationships. But failure of chromosome pairing, or asynapsis, can be caused by genetic and environmental factors as well as by the lack of sufficient homology. All these factors must be considered in any interpretation of chromosome behavior in species-hybrids.

If two species can be crossed and produce a hybrid with normal somatic development, the parental genomes must be somewhat similar in essential genetic factors. In such hybrids, it seems improbable that differences in genetic constitution *per se* would produce sufficient lack of homology to cause a failure of chromosome pairing although it is possible that such factors may be involved.

Changes in chromosome structure are known to be an important factor in changing the homology of chromosomes in different individuals or species. Segmental interchange, inversions and loss or duplication of chromosome segments may change the chromosome structure of different species to such an extent that no two chromosomes in the species-hybrids are completely homologous. Slight changes would not inhibit regular pairing but the hybrid might be partially or completely sterile owing to segmental deficiency following random chromosome distribution at meiosis.

Genetic factors affecting chromosome morphology and chromosome pairing are known to occur in a number of species.<sup>1</sup> A single recessive character in *Matthiola incana* increases the length of the chromosomes at meiosis and decreases the degree of pairing. In *Zea*, a factor for "asynapsis" does not prevent pairing at prophase but chiasma formation is inhibited and pairing is reduced at metaphase. A similar condition is found in *Triticum*. Asynapsis, due to chromosome loss or to less definitely determined genetic factors, has been found in *Primula*, *Crepis*, *Datura*, *Viola*, *Nicotiana*, *Sorghum*, *Avena* and *Triticum*.

In the  $F_1$  of *Lolium*  $\times$  *Festuca*, Peto found regular chromosome pairing at meiosis but certain back-cross segregates showed irregular pairing. This behavior was attributed to segregation of factors limiting chiasma formation. The possible action of such complementary factors is also shown in species-hybrids of *Viola*, *Digitalis*,

<sup>1</sup> Huskins, 1932, and Darlington, 1932, Review and lit. cit.; Beadle, 1933; Sapehin, 1933.

*Chrysanthemum* and in certain generic hybrids in the *Gramineae*.

The nature of chiasma formation may be determined also by genetic factors. In *Allium fistulosa* the chiasmata are localized near the spindle fiber constriction, while in *Allium cepa* they are distributed at random. In the  $F_1$  hybrid between these two species the chiasma formation is at random. Back-cross segregates showed approximately equal numbers of plants with either random or localized chiasma (14).

Environmental conditions may play an important part in meiotic behavior of chromosomes. Either high or low temperature may cause a failure of chromosome pairing and the temperature extremes necessary to cause asynapsis are within the range which the plants occasionally might experience in nature. Irregularities in meiosis may be caused also by insects or virus diseases (20). Environmental factors causing meiotic irregularities are doubtless more effective in individuals with weak meiotic pairing, especially in hybrids.

The effect of genetic and environmental factors in chromosome pairing does make necessary some caution in interpreting chromosome relationships, based on chromosome behavior in species-hybrids. But this caution applies primarily to the negative aspects of the analysis. When chromosome pairing does occur, even though it may not occur consistently, there is usually good reason to believe that the pairing chromosomes are at least partially homologous. The conditions of pairing, even in many pure species, seem to be based on a delicate balance between chromosome development and the stage of meiotic development. These timing relationships may be so delicately adjusted that slight changes in genetic constitution, genic balances or environmental conditions may inhibit pairing of chromosomes which are completely homologous or at least sufficiently homologous to pair under favorable genetic and environmental conditions.

There are, however, certain types of chromosome association that are not dependent on chromosome homology. McClintock (24) finds in *Zea* an association of non-homologous chromosome segments, usually confined to cytologically unbalanced forms. The prophase association of non-homologous segments seems to be as intimate as homologous pairing but chiasmata are not formed and the non-homologous association rarely persists until diakinesis.

McClintock suggests that non-homologous prophase pairing is caused by a mechanism by which chromosomes start their pairing and by a force demanding 2 by 2 associations which can supersede that of homologous attraction when these forces are not working in unison. It is possible that some such factors may be involved in the pairing of non-homologous chromosomes in certain triploids and other cytologically unbalanced hybrids.

*Hybrids Between Species With the Same Chromosome Number  
Fertile Species-Hybrids*

Many hybrids between taxonomic species show regular chromosome pairing at meiosis and a high degree of fertility. The parental species in such hybrids presumably differ only in genetic constitution although minor structural changes may be involved. Regular pairing of the chromosomes at meiosis and a high degree of fertility have been found in certain species-hybrids of *Triticum*, *Ribes*, *Prunus*, *Fragaria*, *Salix*, *Vitis*, *Rhododendron*, *Platanus* and *Larix*.<sup>2</sup> In some cases, the  $F_1$  hybrids show a looser association of bivalent chromosomes than is found in the parental species. In hybrids between 14-chromosome wheat species, Aase notes an increase in the number of rod bivalents at the expense of the ring bivalents. Darlington has analyzed this hybrid in terms of chiasma frequency per bivalent and finds a frequency of 2 for the  $F_1$  as compared with 2.3 and 2.4 for the parents.

Hybrids between Asiatic or European species with American species are especially interesting because they afford an opportunity to study the effects of geographic isolation in species formation. Crosses between the European *Vitis vinifera* and the American *Vitis labrusca* are fertile and have produced many of our commercial varieties. Although *Platanus occidentalis* and *P. orientalis* must have been separated for thousands of generations, the  $F_1$  hybrid shows normal meiotic divisions and fertility. The  $F_1$  hybrid between Asiatic and European species of *Larix* is fertile and the chiasma frequency of the hybrid is as high as it is in the parental species. Such behavior would seem to indicate that such widely separated species have been differentiated without any

<sup>2</sup> Aase, 1930; Babcock and Navashin, 1930; Darlington, 1930, 1931; Hakansson, 1934; Meurman, 1928; Negrul, 1930; Percival, 1932; Sax, 1930, 1932, 1933; Yarnell, 1930.



fundamental genetic or structural changes in the chromosomes for very long periods of time. Certain species evidently differ only in minor genetic factors and maintain their identity only by isolation.

Crosses between different genera also may produce fertile hybrids.<sup>3</sup> The hybrid between the closely related genera *Sorbus* and *Aronia* shows normal chromosome pairing at meiosis and a high degree of fertility. *Zea mays* crossed with certain varieties of *Euchlaena mexicana* produces an  $F_1$  which has normal meiotic divisions and is fertile. Peto found regular pairing of the meiotic chromosomes in a cross between *Lolium* and *Festuca* although there was some evidence of structural hybridity.

### *Sterile Species-Hybrids*

In certain species-hybrids there is regular chromosome pairing at meiosis but the hybrid is partially or completely sterile. Such sterile species-hybrids have been found in *Primula*, *Aquilegia*, *Pisum*, *Philadelphus* and *Campsis*.<sup>4</sup> In the  $F_1$  of *Primula floribunda*  $\times$  *P. verticillata* the nine chromosomes from each parent appear to pair regularly but little good pollen is formed. More than half of the pollen is sterile in the species-hybrids of *Pisum* and Lutkov attributes this sterility to unfavorable genic combinations following the random distribution of the parental chromosomes to the microspores. The writer suggested that the partial sterility in the species-hybrid of *Campsis* might be caused by structural hybridity involving only small chromosome segments. Random distribution of the chromosomes would result in deficiencies of a chromosome segment in certain microspores with corresponding pollen sterility.

Irregular meiotic divisions and pollen sterility are characteristic features of most species-hybrids. Even when the parental species have the same number of chromosomes there is often considerable irregularity in the meiotic divisions in the hybrids. Such species-hybrids have been described in *Nicotiana*, *Crepis*, *Ribes*, *Rosa*, *Brassica*, *Viola*, *Aegilops*, *Gossypium* and in several hybrids.<sup>5</sup> In

<sup>3</sup> Beadle, 1932; Longley, 1924; Peto, 1934; Sax, 1931.

<sup>4</sup> Lutkov, 1930; Newton and Pellew, 1929; Skalinska, 1932; Sax, 1933.

<sup>5</sup> Babcock and Navashin, 1930; Clausen, 1931; Erlanson, 1929; Goodspeed, 1934; Karpechenko, 1927; Morinaga, 1931; Meurman, 1928; Percival, 1932; Webber, 1934.

the genus *Nicotiana* different crosses between 12-chromosome species show almost all degrees of chromosome pairing. The hybrid between *N. paniculata* and *N. Cavanillesii* shows very little chromosome pairing and the few bivalents formed are loosely paired. On the other hand, *N. paniculata* crossed with *N. solanifolia* produces an  $F_1$  hybrid with nearly normal pairing at meiosis and the bivalents are usually as closely paired as they are in the parental species. Other species-hybrids show an intermediate condition with much variability in the amount of chromosome conjugation. Goodspeed concludes that the degree of chromosome pairing in species-hybrids is an index of genetic relationships in *Nicotiana*.

In all species-hybrids with partial pairing of chromosomes there is great variability in the amount of pairing in different pollen mother cells. This variation is especially striking in crosses between 10-chromosome *Brassica* species where the number of bivalents may range from 1 to 9. Chromosome pairing in partially sterile species-hybrids also seems to be much more dependent on environmental conditions than is the case in pure species. The greater protection afforded the developing female gametophytes may account for the fact that the percentage of fertility is greater for the egg cells than for the pollen in many species-hybrids.

In these species-hybrids with failure of chromosome pairing, there is more or less gametic sterility caused by irregular distribution of the univalent chromosomes. These unpaired chromosomes may pass at random to the poles with the dividing bivalents, in which case they divide at the second meiotic division, or they may become oriented on the metaphase plate and divide after the division of the bivalents and pass undivided to one pole or the other at the second division. In either case, some of the univalents often do not reach the poles in time to be incorporated in the daughter nuclei and, consequently, the microspores are deficient in chromosomes necessary for normal development. When no pairing occurs, the univalents, in rare cases, may not complete the first meiotic division, but divide in the second; or they may divide equationally in both divisions. In either case, diploid gametes may be produced which contain the complete haploid complement of chromosomes from each parent. The union of two such functional

gametes is one means of producing the fertile constant species-hybrids to be described later.

Species-hybrids may be so sterile that no gametes are produced.<sup>6</sup> In a *Philadelphus* hybrid between diploid species, the anther contents disintegrate before the meiotic divisions are initiated. East finds a similar condition in certain male sterile segregates in a *Nicotiana* hybrid. The hybrid between *Mahonia* and *Berberis* has been grown in European and American botanic gardens for more than fifty years but it has never been known to produce flowers, even though the  $F_1$  plant is vigorous. Dermen finds 14 pairs of chromosomes in each of the parental species and 28 chromosomes in the somatic cells of the hybrid. Still greater incompatibility is shown in both varietal and species-hybrids where the embryo may die at an early stage of development.

#### *Hybrids Involving Differences in Chromosome Structure*

Structural hybrids are those in which the parental genomes differ in chromosome structure caused by rearrangement of chromosome segments. It is evident in many genera that species differ in chromosome morphology even though the chromosome numbers are the same. There is also good evidence that structural differences may be involved, even when the genomes of different species appear to be similar in chromosome structure.

The most easily recognized types of structural differences are found in individuals heterozygous for reciprocal translocations of large segments. If Aa and Bb are two non-homologous chromosomes, then segmental interchange will give rise to two new types of chromosomes, Ab and Ba. In individuals heterozygous for the two types of chromosomes, the homologous segments pair at meiosis to produce an association of four chromosomes. At metaphase, these chromosomes usually are associated by terminal chiasmata to form a ring or chain of four chromosomes, no two of which are completely homologous. In certain species, the adjacent chromosomes usually pass to opposite poles at meiosis but in most cases adjacent chromosomes pass to the same pole or to opposite poles at random. When adjacent chromosomes pass to the same pole, the gamete is deficient for a chromosome segment and may not be functional. For example, in the chain Aa-aB-Bb-bA, the

<sup>6</sup> Dermen, 1931; Hollingshead, 1930; McCay, 1933; Sax, 1931.

segregation of Aa-aB and Bb-bA to opposite poles will produce microspores deficient for segments b or a.

Segmental interchange has been found in *Datura*, *Zea*, *Pisum*, *Campanula*, *Rhoeo*, *Tradescantia*, *Aucuba*, *Oenothera*, *Godetia*, *Clarkia*, *Hypericum*<sup>7</sup> and other genera. Complete rings involving all chromosomes are found in certain species of *Oenothera* and *Hypericum* and in *Rhoeo*. In these cases, the ring formation is perpetuated by a balanced lethal system which prevents the production of individuals homozygous for chromosome structure. In *Oenothera* these structural hybrids are also heterozygous in genetic constitution but segregates occasionally occur when further interchange occurs between chromosomes of opposite complexes. In other cases, however, different strains or different species may be homozygous for segmental interchange chromosomes and the rings are formed only when crossing brings together different structural genomes.

Different species may differ in chromosome structure even though the differences do not produce the rings and chains in the  $F_1$  hybrids. An extreme type of structural differentiation has been found in a cross between *Crepis divaricata* ( $n=4$ ) and *C. dioscorides* ( $n=4$ ). At meiosis there is variable pairing in  $F_1$  with the production of 0-4 bivalents (29). Some of the bivalents separate with difficulty; chromatids often break; fragments are produced; and occasionally a chromosome is found with two fiber attachments. Müntzing attributes this behavior to pairing of similar segments in structurally different chromosomes followed by crossing-over in the homologous segments. Similar observations have been made in species-hybrids of *Paeonia* by Stebbins (unpublished). If the parental genomes differ only in small or interstitial chromosome segments, the pairing may be regular, but random distribution of bivalents might produce segmental deficiencies which would cause partial sterility. There is some evidence of such behavior in certain partially or completely sterile species-hybrids. The indirect evidence from chromosome structure in related species supports the hypothesis that structural changes have been an important factor in the origin and differentiation of certain species (4).

<sup>7</sup> Sharp, 1934, lit. cit.

*Hybrids Between Varieties or Species With Different  
Chromosome Numbers*

Hybrids involving differences in the chromosome numbers of the parental gametes are of two kinds, autopolyploids and allopolyploids. In the first type, one or both of the parental gametes contain two or more sets of chromosomes which are essentially similar in genetic constitution and morphology. In the allopolyploid hybrids, the polyploid parent or parents produce gametes containing two or more sets of chromosomes which are only remotely homologous. Such allopolyploid numerical hybrids always involve genetic differences, and in some cases structural differences, in the parental chromosomes. It is obviously difficult to classify all numerical hybrids into these two types because intermediate conditions are found in some hybrids.

*Autopolyploid Hybrids*

The simplest polyploid hybrids are varietal triploids, derived from the union of a normal haploid gamete and an exceptional diploid gamete produced by a diploid plant. Crosses between diploids and tetraploids belonging to the same, or to closely related, species also give rise to triploids with three sets of chromosomes or genomes which are essentially identical. Autotriploids have been found in many different genera of plants. The cytological work on such triploids has been reviewed in a recent paper by East (13). Since all three genomes are identical or very similar in such triploids, one might expect pairing between the homologous chromosomes to form trivalents. Complete sets of trivalent chromosomes are common in triploid *Datura*, *Canna* and *Hyacinthus*, and trivalents are usually formed in *Lycopersicum* and *Zea*. Varying numbers of bivalents, univalents and trivalents are found in triploid *Prunus*, *Petunia*, *Pyrus* and *Tradescantia* but the total number of bivalent and trivalent chromosomes is usually equal to the basic chromosome number for the genus. Trivalent association is partially dependent on chiasma frequency. If the chiasma frequency is low, only two of the three chromosomes may pair, leaving the third chromosome as a univalent. In all cases, the chiasma frequency is greater in the triploid than in the diploid.

At the reduction division of the triploid, there is a tendency for the chromosomes of two genomes to pass to opposite poles and for

a random distribution of the chromosomes of the third genom, whether they are present as univalents or combined as trivalents. At the second meiotic division all chromosomes divide and the microspores receive from  $1n$  to  $2n$  chromosomes, the frequencies approaching those expected in a normal frequency distribution. Usually, however, there is some irregularity in division and some chromosomes do not reach the poles in time to become incorporated in the daughter nuclei. If a microspore receives a complete set of chromosomes or a complete genom plus additional chromosomes, the microspore usually develops and produces a functional pollen grain; but a microspore deficient for any one chromosome of the genom usually does not undergo nuclear division and sterile pollen is produced. According to King, most of the pollen sterility in triploid *Tradescantia* can be attributed to chromosome deficiency.

When "triploids" are crossed with diploids, there is usually a great excess of segregates with  $2n$  or  $2n + 1$  or  $2$  chromosomes, especially if the triploid is used as the pollen parent. The aneuploid segregates with intermediate chromosomes have a lower viability and fertility and tend to be eliminated in natural selection. The rapid elimination of the aneuploid types can be attributed to selective fertilization, selective elimination of zygotes and lack of favorable chromosome balance in aneuploid segregates.

An exceptional type of chromosome pairing is found in certain triploids of *Rubus*, *Fragaria* and *Nicotiana*. In *Fragaria*, for example, Yarnell finds that the 21 chromosomes of the triploid form 10 pairs of bivalents plus a univalent, or 9 pairs plus a trivalent, at the first meiotic division. East also finds some evidence of non-homologous pairing in a triploid of *Nicotiana Tabacum*.

More complex types of autopolyploid hybrids have been described in several genera. In a hybrid between a diploid species of *Prunus* and a hexaploid species, Darlington found 16 pairs of chromosomes. The 8 chromosomes contributed by the diploid, pair with 8 chromosomes from the hexaploid, and the two remaining genomes of the hexaploid pair with each other. Somewhat similar cases were found in a *Papaver* hybrid by Ljungdahl and in a *Betula* hybrid by Helms and Jørgensen. A somewhat more complex case in *Chrysanthemum* is described by Shimotomai. In a cross between an octoploid and a tetraploid species, the  $F_1$  had



27 bivalents, attributed to pairing within parental genomes (18+18) 1 (9+9).

Pairing between genomes from one parent only has been found in a species-hybrid of *Crepis* by Collins and Mann and in a *Zea-Tripsacum* cross by Mengelsdorf and Reeves. The two genomes contributed by the tetraploid parent pair with each other, leaving the haploid contribution from the other parent unpaired. Partial or variable pairing among the chromosomes contributed by one of the parents is found in other species-hybrids. A detailed summary of the various types of polyploid hybrids is presented in tabular form in Darlington's "Recent advances in cytology."

### *Allopolyploids*

Typical examples of allopolyploidy are found in species-hybrids of *Triticum*, *Nicotiana*, *Fragaria*, *Gossypium* and other genera. In crosses between Emmer wheats with 14 pairs of chromosomes and Vulgare wheats with 21 pairs of chromosomes, the  $F_1$  meiosis shows 14 bivalents and 7 univalents. The bivalents divide regularly but the univalents lag behind and divide equationally as the bivalent homologues reach the pole. At the second meiotic division the 14 bivalent homologues divide equationally and the univalents are segregated at random, without dividing, to either pole. Occasionally, univalents are lost at either the first or second meiotic division. The gametes produced contain from 14 to 21 chromosomes. About 20 per cent of the pollen grains are aborted but nearly all the egg cells seem to be functional. In  $F_2$  and subsequent generations, segregates with intermediate chromosome numbers are eliminated. The segregates with 14 pairs of chromosomes resemble the Emmer parent, while the 21-chromosome segregates resemble the Vulgare parent. *Aegilops cylindrica* crossed with Vulgare wheats produces an  $F_1$  with 7 bivalents but when crossed with the Emmer wheats, there is no pairing in  $F_1$ . The analysis of these and other hybrids indicates that the Vulgare wheats contain three different genomes of 7 chromosomes each.<sup>8</sup>

Crosses between certain 12- and 14-chromosome species of *Nicotiana* also indicate that allopolyploidy is a factor in species differentiation in this genus.<sup>9</sup> *Nicotiana sylvestris* ( $n=12$ )

<sup>8</sup> Sharp, 1934, pp. 366, lit. cit.

<sup>9</sup> Goodspeed, 1934, lit. cit.



crossed with *N. tomentosa* ( $n=12$ ) produces an  $F_1$  with no pairing at meiosis but either of these species crossed with *N. Tabacum* ( $n=24$ ) produces a hybrid with 12 univalents and 12 bivalents at meiosis. Clausen concludes that the *Tabacum* genom consists of two subgenoms, one homologous with the *N. sylvestris* genom and the other with the *tomentosa* genom. This hypothesis is verified by further analysis of a triplex hybrid. Kostoff crossed *N. sylvestris* with a form of *N. tomentosa* (Rusbyi, according to Kostoff, but *tomentosiformis*, according to Goodspeed) and obtained no pairing in  $F_1$ . A few diploid pollen grains were produced which functioned in the cross between the  $F_1$  and *N. Tabacum*. In the triple hybrid obtained, there were 24 bivalents at meiosis, produced by the pairing of the two genoms from the diploid pollen grain and the corresponding genoms of *Tabacum*.

#### *Amphidiploid Hybrids*

Amphidiploid hybrids are derived from sterile species or generic hybrids by doubling of the chromosome number. In the sterile hybrid the chromosomes may not pair, or if bivalents are regularly formed, the segregation of parental chromosomes produces unviable combinations. In the amphidiploid, each parental genom is duplicated and the chromosomes of similar genoms pair together. In most cases, bivalent pairing occurs; each gamete receives a complete genom of each parental type; fertility is restored; and the hybrids breed true to type. Chromosome doubling may occur either in the soma or in the gametes of the original hybrid.

In 1917, Winge suggested that many of the polyploid species in nature were allopolyploids derived from species-hybrids. This suggestion has been amply confirmed during the past ten years. The first complete analysis of an amphidiploid hybrid was made by Clausen and Goodspeed in 1925. *Nicotiana Tabacum* ( $n=24$ ) crossed with *N. glutinosa* ( $n=12$ ) usually produces an  $F_1$  with irregular chromosome pairing and a high degree of sterility but a few  $F_1$  plants were obtained which were fairly fertile and bred true. These plants had 36 pairs of chromosomes at meiosis, 24 pairs contributed by one parent and 12 by the other. The origin of this constant hybrid, *N. digluta*, was attributed to chromosome doubling in the egg cell. The amphidiploid hybrid *Primula Kewensis* originated as a bud sport from a partially sterile species-

hybrid. The generic hybrid *Raphanobrassica* was produced by chromosome doubling in  $F_1$  gametes. In the cross between *Raphanus* and *Brassica*, Karpechenko found that the 9 chromosomes contributed by each parent failed to pair in  $F_1$ , but some diploid gametes were formed. The union of such gametes produced plants with 18 pairs of chromosomes which were fertile and constant.

Amphidiploid hybrids have been described in many other genera including *Rosa*, *Solanum*, *Digitalis*, *Aesculus*, *Triticum*, *Saxifrage*, *Spartina*, *Brassica*, *Galeopsis*, *Phleum*, *Viola*, *Crepis*, *Salix*, *Fragaria* and *Gossypium*.<sup>10</sup>

In several cases, it has been shown that Linnaean species are amphidiploids and the parental species have been identified. In fact, Müntzing (1930) has been able to synthesize *Galeopsis tetrahit*, a tetraploid species, by crossing two diploid species, *pubescens* and *speciosa*. The  $F_1$  was almost completely sterile but did produce a triploid  $F_2$  plant which, when crossed with one of the parents, produced a tetraploid plant indistinguishable from *G. tetrahit*. *Spartina Townsendii* and *Pentstemon neotericus* are other examples of amphidiploids in nature whose origin is well established by cytological analysis.

Many of the experimentally produced amphidiploids are fertile but some of them are more or less sterile. The degree of fertility seems to be correlated with the degree of differentiation of the genomes of the parental species. If only remote homologies exist in the two genomes, pairing is restricted to homologous chromosomes from each genome, only bivalents are formed and the hybrid is fertile; but if the parental genomes are not so completely differentiated, then multivalent chromosome associations or random pairing between the chromosomes from different genomes, will cause irregularities in meiosis leading to partial sterility. Thus, there is generally a negative correlation between the fertility of the diploid  $F_1$  and the amphidiploid hybrid but sterility may be caused by complementary genetic factors in some cases.

There is other less direct evidence which indicates that many species, genera and even larger taxonomic units have originated

<sup>10</sup> Lit. cit. Winge, 1932; Darlington, 1932; Sharp, 1934. More recent work on *Gossypium* by Skorsted, 1934, and Webber, 1934; on *Pentstemon* by Clausen, 1933; and *Erophola* by Winge, 1933.

as amphidiploids. An outstanding example is found in a subfamily of the *Rosaceae*.<sup>11</sup> All genera of the *Pomoideae* have a basic chromosome number of 17 but the basic number for most other genera of the *Rosaceae* is 7 or 8. In diploid genera of the *Pomoideae* there is frequently considerable "secondary pairing" of chromosomes, suggesting remote homologies within the basic genom of 17 chromosomes. The genetic behavior of *Malus* is complex. These various lines of evidence suggest that the *Pomoideae* are allopolyploids, derived from remote ancestral types with 8 and 9 chromosomes, which in turn may have been derived from species or genera with 7 pairs of chromosomes.

Lawrence (21) finds in many species some secondary association of bivalents at meiosis, suggesting some homology within the chromosomes of each parental complement, sufficient to cause some attraction but not enough to cause chiasma formation and true multivalent pairing. Such secondary pairing, together with a comparison of basic numbers in related genera, may be of value in determining the original basic chromosome number in many genera.

#### *Chromosome Behavior in Hybrids as an Index of Species Relationships*

The cytological analysis of species-hybrids has been of value in determining the relationships and origin of many species of plants. Species which produce hybrids with regular meiotic pairing and normal fertility appear to be differentiated primarily by differentiation of genetic factors. Such species retain their identity only by geographic or physiological isolation.

Partial and variable pairing in diploid species-hybrids can be attributed to structural differences in the parental genomes or to the action of different genetic factors which inhibit chromosome pairing. Structural changes in the chromosomes seem to be an important factor in species differentiation. Such structural changes may in themselves produce morphological variation; and since these changes often inhibit the production of fertile hybrids, they may provide the necessary isolation for the development of diverse species by further accumulation of character differences by gene mutations. Structural changes in chromosomes have been an

<sup>11</sup> Moffett, 1933; Sax, 1933.

important factor in species differentiation in *Crepis* and other genera and seem to be associated with genetic differentiation in the Conifers.

Autopolyploidy may be a factor in species differentiation either as a direct result of chromosome duplication or by the partial isolation of the polyploid from the diploid, permitting independent development of specific differences. The allopolyploids, however, are of much greater importance in the origin of species and genera. Crossing of distinct species of plants followed by chromosome doubling is known to occur in nature and has occurred frequently under experimental conditions during the past ten years. It is of interest to note that many of our cultivated plants are of known or supposed allopolyploid origin. These include wheat, oats, cotton, apples, pears and tobacco. Allopolyploidy has been an important factor in the origin of species and of even larger taxonomic units. Anderson (2) suggests that certain angiosperms may have been derived from species of primitive gymnosperms by allopolyploidy.

Gene mutation, structural changes of the chromosomes, hybridization and polyploidy have been involved in the origin and evolution of species of plants. Cytological analyses of species and species-hybrids have shown which of these factors, or combination of factors, have been responsible for species differentiation in many different genera.

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## GLOSSARY

*Selected by the editors from Darlington's "Recent Advances in Cytology"*  
*Aneuploid*, having an uneven multiple of the basic number of chromosomes through purely numerical aberration—an unbalanced polyploid.

*Balance*, the condition in which the genes are adjusted in proportions which give satisfactory and normal development of the organism.

*Basic Number*, the supposed number of chromosomes found in the gametes of a diploid ancestor of a polyploid.

*Bivalent* (v. Univalent).

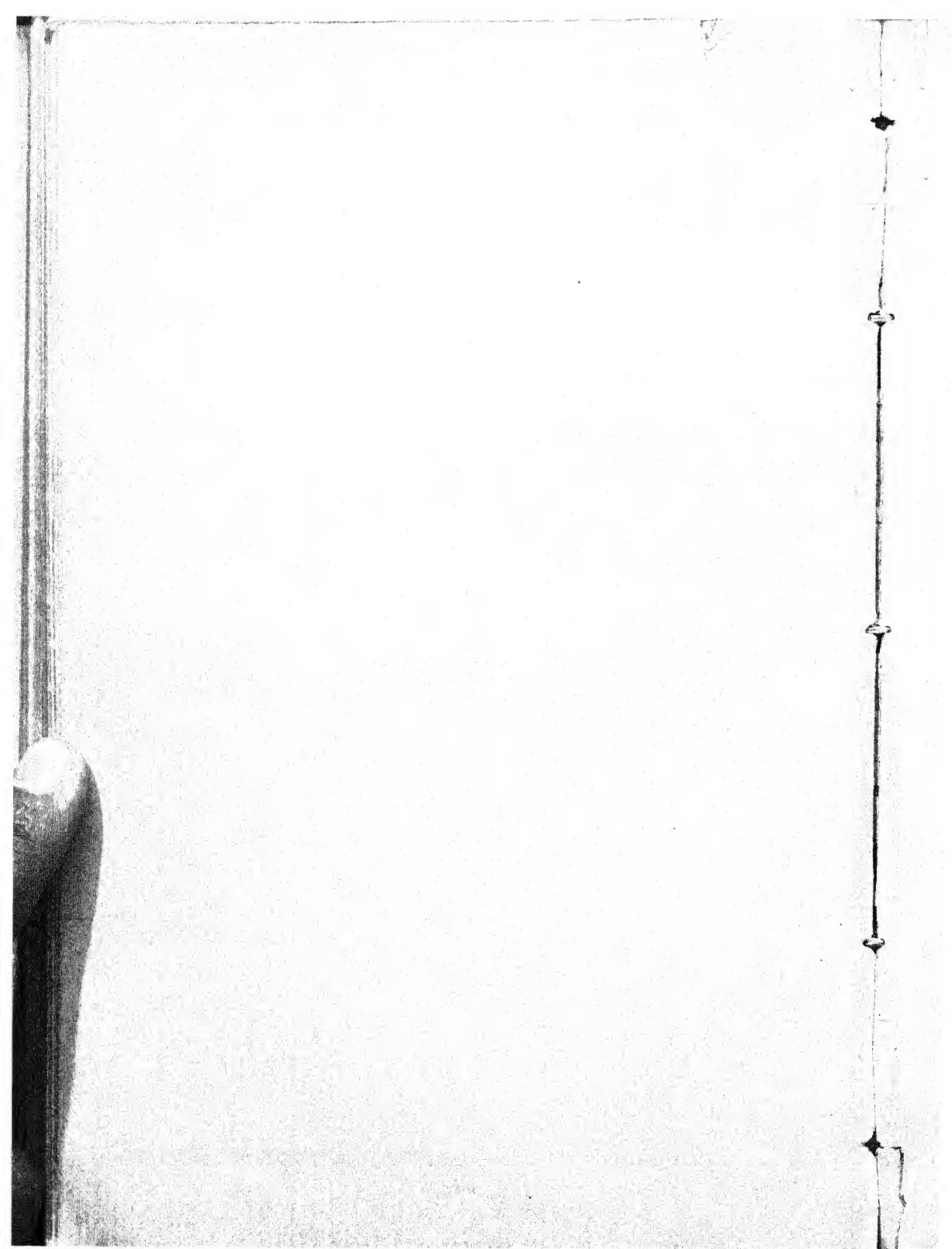
*Chiasma*, -ta, an exchange of partners in a system of paired chromatids.

*Chromatid*, a half chromosome between early prophase and metaphase of mitosis and between diplotene and the second metaphase in meiosis, after which stages, i.e., during an anaphase, it is known as a daughter-chromosome.



- Chromomeres*, the smallest particles identifiable by their characteristic size and position in the chromosome thread.
- Crossing-over*, the exchange of corresponding segments between corresponding chromatids of different chromosomes; a process inferred genetically from the reassociation of linked factors in Mendelian hybrids and cytologically from the reassociation of parts of chromosomes in structural hybrids.
- Deficiency*, loss or "deletion" of a segment of a chromosome from the diploid complement.
- Diakinesis*, the last stage in the prophase of meiosis—immediately before the disappearance of the nuclear membrane.
- Diploid*, (a) the zygotic number of chromosomes ( $2n$ ) as opposed to the gametic or haploid number ( $n$ ); (b) an organism having two sets of chromosomes ( $2\times$ ) as opposed to organisms having one (haploid), three (triploid) or more sets ( $\times$ ,  $3\times$ , etc.).
- Duplication*, the occurrence of one segment of a chromosome twice in the same complement.
- Genome*, a chromosome set.
- Interchange*, an exchange of non-homologous segments of non-homologous chromosomes.
- Inversion*, the reversal of the linear sequence of the genes in one segment of a chromosome relative to an adjoining segment.
- Meiosis*, a form of mitosis in which the nucleus divides twice and the chromosomes once. The prophase of meiosis is the prophase of the first of the two divisions.
- Metaphase*, the stage of mitosis or meiosis in which the chromosomes lie in a plane at right angles to the axis of the spindle and half-way between the poles.
- Pairing of Chromosomes*, the coming together of homologous chromosomes during cell-division.
- Polyloid*, an organism with more than two sets of homologous chromosomes. The terms used are triploid, tetraploid, pentaploid, and so on. Higher multiples are best referred to as  $14\times$ ,  $22\times$  and so on.
- Prophase*, the stage in mitosis or meiosis from the appearance of the chromosomes to metaphase.
- Reduction Division*, formerly applied to the one of the meiotic divisions at which a particular author thought reduction and segregation occurred.
- Rings*, chromosomes associated end to end in a ring, usually by terminal chiasmata.
- Segregation*, the separation of chromosomes of paternal and maternal origin at meiosis and the separation of the differences observed genetically.
- Tetraploid* (v. Polyloid).
- Triploid*, an organism having three sets of chromosomes.
- Univalent*, a body at the first meiotic division corresponding with a single chromosome in the complement; especially when unpaired. Bivalent, Trivalent, etc., are associations of chromosomes held together between diplotene and metaphase of the first division by chiasmata.





# THE BOTANICAL REVIEW

VOL. I

APRIL, 1935

No. 4

## PHYSIOLOGIC SPECIALIZATION OF THE PARASITIC FUNGI<sup>1</sup>

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The discovery by Eriksson (15) of the existence of specialized races of rusts is one of the most important developments in plant pathology. His demonstration that the morphological species of *Puccinia graminis* Pers., *P. glumarum* (Schmidt) Erikss. & Henn., *P. dispersa* Erikss. & Henn. and *P. coronata* Cda. consist of subdivisions which can be distinguished from one another by their ability to grow on some hosts and not on others, has stimulated many workers to investigate the extent and significance of host specialization among the various groups of parasitic fungi. In 1918, I (59) published a fairly complete review of the field, listing 174 papers which described results with the rusts, powdery mildews, downy mildews and other fungi. Since then, through studies of many investigators, the literature on this subject has greatly increased.

The primary basis for evidence of physiologic specialization is the behavior of different collections of parasitic fungi on particular hosts. Recognized hosts of a morphological species of fungus may be infected by some collections and not by others. If conidia of *Erysiphe graminis* DC. from wheat are sown on wheat, barley, oats and rye, the wheat is infected but not the other three cereals. If conidia from barley are sown on the four cereals, only the barley becomes infected. Thus, the mildews on wheat and barley are clearly differentiated from each other by their ability to establish themselves on definite hosts.

Obligate parasitic fungi, such as the rusts and powdery mildews, must be cultured on living plants, and one of the problems is the selection of suitable hosts for differentiation of specialized races,

<sup>1</sup> Brooklyn Botanic Garden Contributions No. 71.

if such exist. Races may also be distinguished by the type and degree of infection. Stakman and Levine (79) have developed a method for detecting physiologic forms of *Puccinia graminis*, which has come into general use. Six types of infection are recognized: 0—immune; 1—very resistant; 2—moderately resistant; 3—moderately susceptible; 4—very susceptible; X—heterogeneous. Different degrees of infection of most of these types serve further to distinguish the reaction of the forms. Differences have also been noted in rate of development of the telial stage of rusts on definite hosts by various collections. Sometimes growth on seedlings and adult plants may also serve to distinguish races.

Many parasitic fungi, such as smuts and various species and genera of the Ascomycetes and Imperfect Fungi, however, may be cultured on laboratory media, and it is possible to distinguish strains or races by differences in their growth characteristics. Such strains are distinguished as "cultural races" from the "pathogenic races," which are differentiated by their capacity for infecting living plants. In culture, it is possible to observe the influence of various substances, such as sugars and other chemicals, on rate and extent of growth. Character of growth, its consistency, whether uniform or sectoring, topography of the surface, color, etc., are all characters which may be used for distinguishing between different strains.

The most extensive studies on physiologic specialization have been made on various parasites of the cereals—rusts, smuts and powdery mildews. The reasons are rather obvious. For one thing, each of the main groups of cereals consists of a large number of varieties which have been derived from many sources and these differ widely in their various agronomic and physiologic characters. Further, the cereals are easily grown and subjected to varied experimental conditions. Finally, the economic importance of cereal crops is undoubtedly a major factor. The seriousness of the various rusts, smuts and other diseases of these crops has led to extensive investigation in all phases of their pathology.

#### PHYSIOLOGIC SPECIALIZATION IN THE CEREAL RUSTS ✓

Eriksson (15) separated *Puccinia graminis* into six "formae speciales": *avenae*, *secalis*, *tritici*, *ariae*, *agrostidis* and *poae*. He further recognized *P. phlei-pratensis* as a distinct species. *P. glumarum*

was found to consist of five specialized forms: *tritici*, *secalis*, *elymi*, *agropyri* and *hordei*. *P. dispersa* consisted of four specialized forms: *secalis*, *agropyri*, *bromi* and *tritici*. Finally, *P. coronata* was separated into six specialized forms: *avenae*, *alopecuri*, *festucae*, *lolii*, *calamagrostis* and *melicae*. Investigations of recent years, however, have resulted in the subdivision of many of these "formae speciales" into secondary groups. In 1917, Stakman and Piemeisel (83) discovered that *Puccinia graminis tritici* Erikss. and Henn. consisted of at least more than one specialized race or "physiologic form." Stakman and Levine (79), in 1922, announced 37 physiologic forms, and in 1934, Stakman, Levine, Cotter and Hines (82) mentioned 127. To this number, Tu (88) has added 6 additional ones for China. According to Mains (40), *Puccinia rubigo-vera* (DC.) Wint. consists of 56 races, based in part upon the relation between telial and aecial hosts. One of these races, *P. rubigo-vera tritici* (Erikss. and Henn.) Carleton, is further separated into 53 sub-groups or physiologic forms.

Some of the most significant advances on physiologic specialization of cereal rusts are given in the accompanying table.

#### GEOGRAPHICAL DISTRIBUTION OF RUST RACES

Standardized methods in the study of biologic forms of rusts have been developed. Stakman and Levine (79) introduced the use of twelve varieties of wheat for differentiating the forms of *Puccinia graminis tritici*. Mains and Jackson (43) employed eleven varieties of wheat in the study of biologic forms of *P. rubigo-vera tritici*. Special varieties and strains of rye, oats and barley have also been employed. Use of the same set of differential hosts in different countries has made it possible to compare the results of studies on physiologic specialization.

✓ Distribution of physiologic forms of *Puccinia graminis tritici* has been studied by a number of workers. Recently, Stakman, Levine, Cotter and Hines (82) recorded 82 physiologic forms, identified from more than 8,000 cultures. Form 36 occurred most commonly and was most widely distributed and was found in 11 States. Form 38 was isolated from collections made in 7 States. Some forms were collected only once. Newton and Johnson (49) stated 41 physiologic forms have been isolated in Canada between 1919 and 1930. Different physiologic forms predominated in dif-

## PHYSIOLOGIC FORMS IN THE CEREAL RUSTS

Fungus	No. of Races	Year	Authority	Country
<i>Puccinia anomala</i> ....	2	1930	Mains (38)	U. S.
	4	1931	Brown (7)	Canada
	8	1931	Hey (29)	Germany
<i>Puccinia coronata avenae</i> .....	2	1919	Hoerner (30)	U. S.
	4	1927	Parson (54)	U. S., Canada
	4	1930	Peturson (55)	Canada
	33	1930	Frenzel (19)	Germany
	33	1933	Murphy (47)	U. S.
<i>Puccinia glumarum</i> ..	4	1930	Allison and Isenbeck (2)	Europe
	5	1931	Wilhelm (94)	Europe
<i>Puccinia graminis avenae</i> .....	4	1923	Stakman <i>et al.</i> (80)	U. S.
	5	1925	Bailey (4)	U. S., Sweden
	5	1929	Waterhouse (91)	Australia
	5	1930	Tedin (84)	Sweden
	2	1931	Verwoerd (89)	South Africa
	9	1933	Gordon (24)	Canada
<i>Puccinia graminis secalis</i> .....	2	1923	Levine and Stakman (37)	U. S.
	14	1932	Cotter and Levine (13)	U. S.
	2	1931	Verwoerd (89)	South Africa
<i>Puccinia graminis tritici</i> .....	1	1917	Stakman and Piemeisel (83)	U. S.
	37	1922	Stakman and Levine (79)	U. S.
	8	1929	Waterhouse (91)	Australia
	2	1931	McDonald (44)	Central Africa
	8	1931	Verwoerd (89)	South Africa
	41	1932	Newton and Johnson (49)	Canada
	6	1934	Tu (88)	China
	127	1934	Stakman <i>et al.</i> (82)	World
<i>Puccinia rubigo- vera tritici</i> .....	12	1926	Mains and Jackson (43)	U. S.
	4	1928	Scheibe (75)	Central Europe
	2	1929	Waterhouse (92)	Australia
	8	1930	Scheibe (76)	Central Europe
	3	1930	Wellensiek (93)	Netherlands
	7	1931	Dodoff (14)	Bulgaria
	6	1931	Tscholakow (87)	Europe
	1	1930	Johnston (34)	U. S.
	26	1932	Johnston and Mains (35)	U. S.
	10	1932	Radulescu (58)	Rumania
	53	1933	Mains (40)	World
	7	1928	Stakman <i>et al.</i> (78)	U. S.

ferent years; some forms were commonly isolated while others were rare.

Waterhouse (91) records the isolation of 8 biologic forms in Australia, 7 of which were restricted to that country while one was identical with a physiologic form found in the United States. Tu (88) isolated 6 forms in China, 2 of which are identical with American forms, and 4 are new. McDonald (44) and Verwoerd (89), in Africa, have identified their physiologic forms with similar ones in the United States.

Mains and Jackson (43), in 1926, described 12 biologic forms of *Puccinia rubigo-vera tritici*. Scheibe (75, 76), in 1928 and 1930, isolated 12 forms, 11 of which differed from those of Mains and Jackson. Dodoff (14), in 1931, isolated 7 forms in Bulgaria, only one of which was new.

Frenzel (19), in Europe, isolated 33 biologic forms of *Puccinia coronata avenae* out of only 27 collections. Murphy (47), during the period 1927 to 1932, isolated 33 physiologic forms of crown rust of oats from 533 collections obtained in 29 States in the United States, 3 States of Mexico and 3 Provinces of Canada.

Rudorf and Job (71) have found that the physiologic forms of *Puccinia graminis tritici* in the Argentine are different from those in North America. On the other hand, of the 5 forms of *P. rubigo-vera* isolated, 2 seemed to be identical with the North American forms 5 and 9.

#### PHYSIOLOGIC SPECIALIZATION IN THE SMUTS

It is interesting to note that in my review of physiologic specialization, published in 1918 (59), no experimental evidence of this phenomenon in the smuts was recorded. In fact, it was not until 1921 that Zillig (95) definitely established the existence of specialized races in *Ustilago violacea* (Pers.) Fuck. by inoculation experiments. The first experimental evidence of specialization in cereal smuts was published in 1924 when Faris (17) described five races of *U. hordei* (Pers.) K. & S., separated by their reaction on certain varieties of barley, and Reed (60) published data that showed that both the loose and covered smuts of oats were differentiated into distinct races. The latter used spores from each species of smut of oats, collected in Missouri and in Wales, for inoculating a series of oat species and varieties. Distinct differences



in infection capacity of the collections from the two countries were observed. His results were confirmed in the following year by Miss Sampson (72). In 1927, Reed (61) described two new races of loose smut of oats occurring on red oat varieties, which hitherto had shown a high degree of resistance to the smuts; one of the new races attacked Fulghum, as well as a number of oat varieties be-

<i>Fungus</i>	<i>No. of Races</i>	<i>Year</i>	<i>Authority</i>
<i>Sphacelotheca sorghi</i> (Link) Clint. ...	2	1927	Tisdale <i>et al.</i> (85)
	5	1932	Melchers <i>et al.</i> (46)
<i>Sorosporium reilianum</i> (Kuehn)			
McAlp. ....	2	1927	Reed <i>et al.</i> (66)
<i>Tilletia laevis</i> Kuehn, and <i>Laevis</i> Trit.			
<i>T. tritici</i> (Bjerk.) Wint. ....	4	6 1928	Reed (63)
	3	2 1927	Rodenhisser and Stakman (69)
	2	3 1928	Gaines (20)
		1 1930	Reichert (67, 68)
	2	3 1931	Holton (31)
	6	4 1931	Bressman (6)
	5	1 1931	Aamodt (1)
	2	5 1933	Gaines and Smith (21)
	6	7 1933	Flor (18)
	7	1934	Melchers (45)
	<i>Ave. Lev.</i>		
<i>Ustilago avenae</i> (Pers.) Jens., and <i>U. levis</i> (K. & S.) Magn.	2	2 1924	Reed (60)
	2	2 1925	Sampson (72)
	2	1927	Reed (61)
	11	5 1929	Reed (64)
	2	1 1932	Reed and Stanton (65)
	1	1 1929	Sampson (73)
<i>Ustilago hordei</i> (Pers.) K. & S. ...	5	1924	Faris (17)
<i>Ustilago tritici</i> (Pers.) Jens. ....	4	1930	Grevel (26)
<i>Ustilago zeae</i> (Beck.) Ung. ....	7	1926	Christensen and Stakman (11)

longing to the group of common oats. The second race was rather sharply confined to Red Rustproof and its close relatives, although it was able to pass on to the common oat variety, Canadian. Reed (64), Reed and Stanton (65), Sampson (73), and Nicolaisen (52, 53) have added further evidence of specialization in the oat smuts.

In 1924, Faris (16) suggested, on the basis of some of his experiments, that physiologic specialization occurred in *Tilletia laevis*



Kuehn and *T. tritici* (Bjerk.) Wint. Reed (62), in 1927, demonstrated conclusively that Martin wheat was susceptible to certain collections of bunt and resistant to others; in 1928 (63) his detailed results were published in which 6 races of *T. tritici* and 4 races of *T. laevis* were definitely established. Rodenhiser and Stakman (69), in 1927, published some data indicating the existence of 3 races of *T. laevis* and 2 of *T. tritici*, and in 1928, Gaines (20) and Roemer (70) reported that a collection of *T. tritici* from Washington State differed from another collection from Germany in its capacity for infecting certain varieties of wheat.

Demonstration of specialization in cereal smuts has stimulated many investigators in various places. Some of the results already obtained are presented in tabular form on preceding page.

#### PHYSIOLOGIC SPECIALIZATION IN THE POWDERY MILDEWS AND OTHER FUNGI

There is very extensive literature on specialization in powdery mildews, downy mildews, various Ascomycetes and Fungi Imperfecti which it is impossible even to mention here. A very brief reference to a few papers will indicate some of the progress that has been made:

<i>Fungus</i>	<i>No. of Races</i>	<i>Year</i>	<i>Authority</i>
<i>Albugo candida</i> (Pers.) Rouss ..	5	1934	Togashi and Shibasaki (86)
<i>Albugo ipomoeae-panduranae</i> (Schw.) Sw. ....	2	1928	Ciferri (12)
<i>Albugo tragopogonis</i> (DC.) S. F. Gray .....	6	1927	Pfister (57)
<i>Colletotrichum lindemuthianum</i> (Sacc. & Magn.) Bri. & Cav. ..	5	1928	Budde (8)
	12	1931	Peuser (56)
	34	1932	Schreiber (77)
<i>Erysiphe communis</i> Grev. ....	26	1925	Hammarlund (27)
<i>Erysiphe graminis</i> DC. f. sp. <i>hordei</i> .....	5	1930	Mains and Dietz (42)
<i>tritici</i> .....	2	1933	Mains (39)
<i>Erysiphe horridula</i> Rab. ....	7	1922	Blumer (5)
<i>Helminthosporium gramineum</i> Rab. ....	20	1934	Christensen and Graham (10)
<i>Phyllactinia guttata</i> Lév. ....	6	1925	Hammarlund (27)

One of the particularly significant features in connection with specialization of powdery mildews is the discovery by Mains (39), and Mains and Dietz (42), that *Erysiphe graminis tritici* and *E. graminis hordei* may be further subdivided into physiologic forms, thus paralleling the results obtained with many cereal rusts.

#### MORPHOLOGICAL VARIATIONS IN SPECIALIZED RACES

✓ Extensive investigations have been made on the question as to whether these specialized races may be distinguished by differences in spore size or other morphological characters. Levine (36) published detailed results with the physiologic forms of *Puccinia graminis tritici* and has found minor variations in spore size and shape which seem to be distinctive of definite races. Blumer (5) has distinguished some of the specialized forms of *Erysiphe horridula* by their spore measurements. ✓ Togashi and Shibasaki (86) have separated *Albugo candida* into the varieties *macrospora* and *microspora*, based upon differences in spore size, and they have further found that the first variety consisted of two distinct biologic forms, and the second variety, of three. ✓ Savulescu and Rayss (74) recorded 8 forms of *A. candida* on the basis of minute differences in conidial measurements. ✓ One of the most extensive studies of this character was made by Gäumann (22) on the genus *Peronospora*. He has split up several of the species on the basis of minor variations in spore size and shape. *Peronospora parasitica* (Pers.) Fries, for example, has been separated into 25 groups which he has raised to specific rank.

It is a question to what extent these minor variations in spore size and shape are significant. Many of the differences recorded are exceedingly minute and it may be doubted whether they have value at all comparable to that obtained through inoculation experiments.

The fact that there are these minor differences, however, naturally raises the question as to the distinction between a physiologic race and a morphological species or variety. Apparently, there is no real sharp line of separation. Many species of parasitic fungi can readily be recognized by morphological characters; in other cases, differences are relatively indefinite. Many of the "formae speciales" of *Puccinia rubigo-vera* have been recognized in books as so-called species. Arthur, in his "Manual of the Rusts in United

States and Canada," has called attention to the fact that, in the determination of a rust, one of the most important factors is the correct name of the host. It is very doubtful whether many of the entities listed as species could be accurately identified without knowledge of the plant upon which the material was collected.

INFLUENCE OF ENVIRONMENTAL FACTORS ON THE REACTION OF DIFFERENTIAL HOSTS TO VARIOUS PHYSIOLOGIC FORMS OF RUSTS

Waterhouse (91), in 1929, made the significant discovery that the specific reaction of a host to certain physiologic forms of *Puccinia graminis tritici* varied under winter and summer conditions. In some cases, he found that a given variety was quite susceptible in hot summer months and resistant in winter months to certain specialized forms. Marquis varied from complete susceptibility to forms 46 and 55 during summer to moderate resistance in winter. This variety is a differential host for forms 45 and 55; its usual reaction to 45 is moderate resistance and, to 55, high susceptibility. If, however, the environmental conditions change its reaction with form 55 from susceptibility to moderate resistance, the line of separation between the two forms largely disappears.

Waterhouse (91) found similar variations in the behavior of certain forms of *Puccinia graminis avenae*, *P. triticea* and *P. simplex*. With the latter species, 14 susceptible varieties of barley gave the same results during both summer and winter. However, 8 varieties resistant under winter conditions were susceptible during the summer period.

Gordon (23, 24), in 1930 and 1933, published similar data on certain forms of *Puccinia graminis avenae*. White Russian, Richland, Victory and Joannette showed no significant differences in their reaction to physiologic forms 2, 6, 7 and 8 when grown at four different temperatures from 57.4° F. to 75.4°. The Joannette strain, however, was very resistant to forms 1, 3, 4 and 5 at low temperatures and susceptible at high. Peterson (55), in 1930, found that Red Rustproof oats was resistant to form 4 of *Puccinia coronata avenae* at 57° F. and susceptible at both 70° and 77°. Four other varieties, Green Mountain, White Tartar, Green Russian and Sterilis Selection were fully susceptible at all three temperatures while Ruakura was resistant. Johnson (32) obtained similar results with forms of *P. graminis tritici*. Other en-

viral factors have also been studied and found to have a definite effect.

Goulden, Newton and Brown (25), in 1930, stated that certain wheat varieties showed no essential differences in reaction to 16 physiologic forms of *Puccinia graminis tritici* in the seedling and mature plant stage. A few varieties, however, varied markedly in resistance in the two stages of plant growth.

These results are extremely significant in the problem of differentiating specialized races and they emphasize the fundamental importance of environal factors in the interplay of host and parasite.

In cereal smuts, environal conditions are known to have a determining influence upon infection and it is necessary to supply favorable conditions for infection in order to determine the real reaction of a variety. Much of the work on cereal smuts has been done by sowing seed in the open and depending upon chance combination of favorable environal conditions. Usually, low percentages of infection have resulted and it is doubtful whether much significance can be attached to results based upon slight variations in percentage of infection of varieties under such conditions.

The recognition of the part played by environal factors is of significance in connection with the study of inheritance of disease resistance. Waterhouse (91), in 1929, reported that certain barley crosses gave different results in winter and summer months. Some families which in winter had given him a normal ratio of three resistant to one susceptible, in summer months failed to show the expected segregation. Harrington (28) found that a series of progenies of a cross of Marquillo x Marquis showed susceptibility as dominant with form 21 of *Puccinia graminis tritici* at a high temperature, while at a low temperature resistance was dominant. Mains (41) has also recorded that hybrids between Michigan Amber and Chinese wheats were difficult to classify in their reaction to a race of *Erysiphe graminis tritici* when grown in the spring. In winter time, however, it was comparatively easy to classify the hybrid lines. The Michigan Amber parent was resistant in winter while more or less susceptible in spring.

#### THE CONSTANCY OF SPECIALIZED RACES

It is an important question whether the races, forms, biotypes, etc., which are isolated through reaction of differential hosts, are

true-breeding constant entities. Isolations cultured on living hosts have shown remarkable uniformity over a period of successive generations, frequently covering years of time. The same sets of varieties have been utilized in different parts of the world for differentiating cereal rust physiologic forms and, so far as the particular varieties used are concerned, the forms may be identified in widely separated countries.

It is also interesting to note the ease with which two or more races may be separated from mixed collections of parasitic fungi. Frenzel (19) isolated 33 distinct races of crown rust of oats from 27 different collections. In rust surveys carried out in the United States and Canada, some of the same forms have been isolated year after year, frequently from widely separated localities. The Missouri races of loose and covered smuts have been grown annually for more than twenty years and, throughout that period of time, have shown a constant behavior on given varieties of oats. They have been artificially mixed and used for inoculating oat varieties, and the two species, with their usual capacities for infection, have again been secured. Mixtures of different races of smuts have been collected in the field. One of the best illustrations is the fact that a collection of loose smut was received from Texas and used to inoculate certain oat varieties. As a result of the experiments, the original mixture was found to consist of two distinct new races of loose smut, one capable of infecting Fulghum and the other, Red Rustproof.

In the study of racial specialization, the question of bridging hosts has occupied a prominent place. Such a host was defined as a plant which enabled a specialized race to extend its normal range. A fungus growing on one plant might be able to infect a second slightly and a third not at all. By growing the parasite on the second variety, it was supposed to acquire an ability to pass successfully over to the third. At the present time, however, there seems to be no clear-cut evidence that such a type of adaptation really occurs. There has been no demonstration of extension of range of hosts through acquired pathogenicity. In smuts, a low percentage of infection of a particular variety is sometimes obtained. Spores collected on such a variety and resown sometimes give a very high percentage of infection. Apparently, what has really been done is to separate out two specialized races from the original mixture.

In contrast to the apparent constancy of races on living plants, passing reference may be made to the behavior of races that are isolated on artificial media. Many investigators have published data on cultural behavior of various species of smuts, and marked variation in cultural behavior of the isolations with reference to color, consistency, topography of the surface, rate of growth, sectoring, etc., have been recorded. The significance of these variations is by no means clear. It has been assumed by some to be associated with hybridization and possibly mutation, resulting in the formation of new specialized strains.

Specialized races of cereal rusts are based primarily on cultures of urediospores. These rusts are heteroecious and their complete cycle includes stages on both cereals and barberry or other aecial host. In the life cycle, urediospores are binucleate and give rise to binucleate mycelium. In other words, rust races are characteristically based upon the diploid stages of the parasite.

On the other hand, powdery mildew races are based upon inoculation experiments with conidia and, so far as their position in the life history of the fungus is concerned, conidia are haploid in nature and serve to perpetuate by successive generations the haploid phase of the powdery mildew. Ascospores of mildews have also been used for inoculations. These, however, are also haploid in nature.

In smuts there is a different situation. Young chlamydospores are binucleate and, in some stage of their development, nuclear fusion occurs. When the chlamydospores germinate, a promycelium is formed which gives rise to uninucleate sporidia. However, before infection of the host takes place, fusion of two conidia is necessary although, in the corn smut, so-called solopathogenic lines have been demonstrated by Christensen (9). The inoculum ordinarily used in smuts is the diploid chlamydospore but, during the few hours between inoculation and infection, the parasite passes through the haploid stage back again into the diploid.

#### ORIGIN OF NEW SPECIALIZED RACES

The origin of new specialized forms has always attracted attention. It is evident that specialization is practically a universal phenomenon in parasitic fungi and the number of races that may be isolated appears to be unlimited. So far as cereal rusts are con-



cerned, evidence is accumulating that specialized forms originate on the barberry through hybridization. Newton, Johnson and Brown (50, 51) selfed 8 physiologic forms of *Puccinia graminis tritici* by mixing the pycnial nectar on barberry leaves. Isolations were then made from the aecia and the reaction of the cultures on wheat varieties determined. They found that physiologic form 9, when selfed, gave rise only to form 9; thus it was homozygous. On the other hand, 7 other forms each gave rise to 2 or more biologic forms; in fact, from the selfing of form 53 they obtained 18 different known biologic forms of the rust. These workers have also recorded some data on crossing different forms. A cross between physiologic form 9a and 36 gave, in the  $F_1$  generation, form 17a; in the  $F_2$  generation, physiologic forms 1, 1a, 11, 11a, 15, 17, 17a, 36, 36a, 57a and 85 were secured. Further data are given by Johnson, Newton, and Brown (33).

Stakman, Levine and Cotter (81) made 5 crosses between form 36 of *Puccinia graminis tritici* and *P. graminis agrostidis*. Two of the crosses gave negative results. From one cross, 4 physiologic forms of *tritici* were isolated; from a second, 1 form; and from a third, 9 forms. Three of the forms isolated were hitherto unknown. It is interesting that no physiologic forms of *P. graminis agrostidis* were recovered. Crosses between physiologic forms of *P. graminis tritici* and *secalis* were also studied.

Very few mutations in rust physiologic forms have been recorded. Newton and Johnson (48) reported a color mutation in physiologic form 9 of *Puccinia graminis tritici*; a race with orange color urediospores appeared after six uredinial generations. The new form was identical in its pathogenicity and both forms have since been cultured several years without showing any further changes.

Waterhouse (90), in 1929, also obtained a color variant in one of his cultures of form 34 of *Puccinia graminis tritici*. Stakman, Levine and Cotter (81) observed the appearance of a new form in cultures of form 1 of *P. graminis tritici* after more than thirteen years. It was identified as physiologic form 60 and differed from any other races known at the time. Johnston (34) has also described an aberrant form of *P. triticina*.

The aecial host of rusts has been regarded by many students as a possible meeting-ground for various specialized races and thus



might serve as a bridge for the races to extend their host range. Arthur (3) came to the conclusion that the "barberry acts as a bridging host between each and every other gramineous host." Recent investigations support the view of special importance of the aecial host in physiologic specialization. If physiologic forms are heterozygous in the uredial stage, they may appear uniform and constant as long as cultivation by urediospores is continued. If, however, they pass over on to the aecial host, new combinations, with segregation, resulting in new capacities for infection of uredial hosts may occur. Further, if two forms differing in capacity for infecting uredial hosts hybridize on the aecial plant, new combinations may arise which may later be separated out through the aeciospores and perpetuated by the urediospores.

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## REMARKS ON THE LIFE-HISTORY OF THE RHODOPHYCEAE

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In the Rhodophyceae the fertilized egg never separates from the motherplant but remains in intimate connection with it and by continued growth develops into a so-called gonimoblast. The gonimoblast generates a great many spores (carpospores) which disseminate in the water and give rise to new individuals. The gonimoblast receives practically all its nourishment from the motherplant and a great variety of organs are developed among the Florideae in order to facilitate supply of food to the gonimoblast. For classification of red algae those organs are very important and in a study of the lines of phylogenetical development in the Florideae it is essential to determine carefully the origin and structure of food-supplying organs.

Schmitz (22) early pointed out that sporogenous filaments of the Rhodophyceae connect themselves with nutritive cells in the motherplant and he calls these auxiliary cells. Later on Kylin (13) called attention to the fact that there are different kinds of auxiliary cells: those which are only nutritive cells and those which serve both as food-suppliers and as starting-points of the gonimoblasts. Kylin (14) calls the latter typical auxiliary cells. Typical auxiliary cells are found in the orders Cryptonemiales, Gigartinales, Rhodymeniales and Ceramiales, while in the orders Nemaionales and Gelidiales typical auxiliary cells do not occur but only various nutritive ones are found.

As a characteristic feature of the Nemaionales it can be pointed out that all food which the motherplant gives to the growing gonimoblast goes through the carpogonial branch. In the Nemaionales the gonimoblast is connected with the motherplant only by the carpogonial branch (concerning one exception see further on). The gonimoblast develops directly from the fertilized carpogonium (concerning one exception see further on).

As to development of the gonimoblast in the Nemaionales the following examples will be presented. In *Batrachospermum* the carpogonium, when fertilized, produces a number of filaments



which together form a gonimoblast. All food from the motherplant to the gonimoblast passes through the carpogonial branch whereby cells of the carpogonial branch become rather full of nutriment but do not fuse with each other. In *Nemalion* the carpogonium, after being fertilized, divides by a cross wall into two cells. The upper one gives rise to the gonimoblast, the lower one fuses gradually with the hypogynous cell which in turn fuses with the other cells of the carpogonial branch, and in this way there is formed a fusion cell full of food. Through this fusion cell food is transported from the motherplant to the gonimoblast. It is evident that this cell is of importance in the physiology of nutrition. The cell may be called an auxiliary one but it is not a typical auxiliary cell according to the terminology of Kylin. Wilke and Ziegenspeck (27) call it a carpogonium-auxiliary.

In *Scinaia* there are developed from the hypogynous cell four cells before fertilization which are rich in nutritive material. After fertilization the carpogonial nucleus enters into one of these nutritive cells and there reduction division takes place and four nuclei are formed. One of these nuclei proceeds to the carpogonium from which a gonimoblast develops. The food to the gonimoblast is supplied by the motherplant and passes through the carpogonial branch. There is no other connection between the gonimoblast and its motherplant. Svedelius (24) calls the four nutritive cells auxiliary cells. In my opinion they are, however, not typical auxiliary cells but carpogonium-auxiliaries in the sense of Wilke and Ziegenspeck.

In *Bonnemaisonia* there develops, before fertilization, from the hypogynous cell a bunch of filaments consisting of many nutritive cells. After fertilization there grows out from the carpogonium a gonimoblast which, in the early stage of its development, takes food from the nutritive cells. Thereby the carpogonium fuses with the hypogynous cell and this one in turn fuses with the nutritive cells. Only a small quantity of food is, however, transferred from the motherplant to the gonimoblast through the carpogonial branch and the gonimoblast, therefore, also connects itself with other nutritive cells in the motherplant; the gonimoblast, moreover, will not be fully developed until the food supply through the new connection is sufficiently rich. Thus, in *Bonnemaisonia* there are two different kinds of nutritive cells but there are no typical auxiliary

ones. The hypogynous cell as well as the nutritive cells that are developed from the former are carpogonium-auxiliaries. They serve during the early stage of the development of the gonimoblast. Those nutritive cells which serve in the mature stage of the development cannot be called typical auxiliary cells because the gonimoblast does not begin its development from them but from the carpogonium itself. It is, however, very interesting to note that the gonimoblast is connected with the motherplant in quite a different way than by the carpogonial branch.

*Asparagopsis* has recently been investigated thoroughly by Svedelius (26) who found that it conforms with *Bonnemaisonia* in many respects but also differs in several ways. In *Asparagopsis*, as in *Bonnemaisonia*, there is developed, before fertilization, a group of nutritive cells from the hypogynous cell. After fertilization the carpogonial nucleus proceeds into the hypogynous cell where reduction division occurs. After reduction division the gonimoblast grows out from the hypogynous cell which for food supply connects itself with the above-mentioned group of nutritive cells. Svedelius classifies the hypogynous cell as a typical auxiliary cell but according to my opinion this cell ought not to be called a typical auxiliary cell. I prefer to consider the hypogynous cell and the nutritive cells growing out from it as carpogonium-auxiliaries. During further development of the gonimoblast the cells in the carpogonial branch fuse with one another and with large nutritive cells belonging to the motherplant, forming a large fusion cell from which the gonimoblast then develops. There are similar nutritive cells in *Bonnemaisonia* but here food for the growing gonimoblast passes through a connection which has nothing to do with the carpogonial branch, while in *Asparagopsis* all food to the gonimoblast goes through the carpogonial branch.

In *Atractophora*, just as in *Asparagopsis* and *Bonnemaisonia*, we find a group of nutritive cells coming from the hypogynous cell. Together they form carpogonium-auxiliaries. In *Atractophora* these are, however, scarcely of any importance because the carpogonium, when developing, connects itself with the supporting cell of the carpogonial branch and thus, for its food-supply, enters into direct connection with the motherplant in another way than through the carpogonial branch. The supporting cell is of great physiological importance from an alimentary point of view but it

cannot be designated a typical auxiliary cell because the gonimoblast does not develop from it but from the carpogonium.

In the order Gelidiales there are developed, before fertilization, many food-containing filaments which closely wind round the central cells of the procarps. The gonimoblast grows out directly from the fertilized carpogonium and the growing filaments of the gonimoblast obtain their nutritive material from the food-containing filaments mentioned above. No typical auxiliary cells are to be found in this order. The Gelidiales belong to the diplobiontic Florideae. Reduction division occurs at development of tetraspores which are found on special plants. In Nemalionales reduction division occurs immediately after fertilization, perhaps with the exception of *Liagora tetrasporifera* (see further on). The Nemalionales belong to the haplobiontic Florideae.

In the order Cryptonemiales, Gigartinales, Rhodymeniales and Ceramiales the gonimoblast never develops from the fertilized carpogonium but the latter connects itself in some way or other with a cell in the motherplant and the gonimoblast develops from this, after having received a diploid nucleus. The cell from which development of the gonimoblast occurs is called a typical auxiliary cell by Kylin (14). In all Florideae with typical auxiliary cells direct connection is formed between the gonimoblast and the motherplant, and the carpogonial branch plays no part in transportation of nutritive material. Thus, the food-supply becomes more direct. A typical auxiliary cell never occurs in the carpogonial branch. Before fertilization the auxiliary cell may be rich in nutritive content but it is as frequently devoid of such material and is then not to be distinguished from surrounding vegetative ones.

Kylin (15) points out that there are several kinds of typical auxiliary cells. Formerly three types were distinguished but to these may now be added one more (the Rhodymenia type). Thus, there are the following four kinds of typical auxiliary cells:

(1) The Dumontia type: the auxiliary cells appear in special accessory branches which are developed before fertilization.

(2) The Platoma type: a normal intercalary cell of the motherplant serves as an auxiliary cell.

(3) The Rhodymenia type: the auxiliary cell is already cut off from a daughter cell of the supporting cell before fertilization.

(4) The Ceramium type: the auxiliary cell is cut off from the supporting cell of the carpogonial branch after fertilization.

The four kinds of typical auxiliary cells mentioned above are characteristic of the orders Cryptonemiales (the Dumontia type), Gigartinales (the Platoma type), Rhodymeniales (the Rhodymenia type) and Ceramiales (the Ceramium type), respectively.

The fertilized carpogonium may become connected with the auxiliary cell in various ways. In lower forms belonging to both the Cryptonemiales (*Dumontia*, *Dudresnaya*, *Grateloupia*) and the Gigartinales (*Platoma*, *Furcellaria*, *Agardhiella*, *Rhabdonia*) there are formed long and sometimes branched connecting filaments which transfer diploid nuclei from the carpogonium to many auxiliary cells lying rather remote from the carpogonium. In the higher forms of both the Cryptonemiales and Gigartinales no connecting filaments are formed but the fertilized carpogonium becomes connected directly with an adjacent, previously fixed, auxiliary cell (*Callymenia* and *Callophyllis* in the Cryptonemiales and *Cystoclonium*, *Calliblepharis*, *Hypnea*, *Phyllophora* and *Gigartina* in the Gigartinales). In this case the carpogonium and the auxiliary cell form a unit which is called a procarp. In the orders Rhodymeniales and Ceramiales procarps are always formed and as a rule there is a direct connection between the carpogonium and the auxiliary cell. Only in some of the more primitive species is a short one-celled (seldom two-celled) connecting filament formed (*Antithamnion*, *Callithamnion*, *Spermothamnion*, *Dasya*).

As stated above, typical auxiliary cells serve a double purpose of being both nutritive cells and starting points of gonimoblasts. Typical auxiliary cells never lose their function of being starting points of development of gonimoblasts, but they can lose their nutritive power. Sometimes they have a nutritive function only during the very first stages of the development of the gonimoblasts but are of no importance later on and seldom do not disappear totally. In such cases, there are other cells which succeed them in acting as a food-supplying medium. Some examples are cited below.

*Cryptonemiales.* In *Dumontia* and in *Dudresnaya* the gonimoblasts are comparatively small and food supplied through auxiliary cells is sufficient for their further development. The auxiliary cells do not fuse with the surrounding cells. In *Grateloupia* the

gonimoblast is somewhat larger than in the two above-mentioned species but even here all food is supplied through the auxiliary cell. This cell becomes large but it does not seem to fuse with surrounding vegetative cells. In *Callophyllis* the auxiliary cell is already large and rich in food before fertilization occurs. After fertilization the auxiliary cell develops a number of gonimoblast-filaments which, during the early stages of their development, obtain their food from the auxiliary cell. From the sterile tissue surrounding the procarp there develops, after fertilization, however, a large number of filaments which together form a kind of nutritive tissue into which the filaments of the gonimoblast grow. In its further development the gonimoblast is supplied with food from this nutritive tissue. In the full-grown gonimoblast the auxiliary cell has disappeared totally.

*Gigartinales.* In *Calosiphonia*, *Furcellaria* and *Halarachnion* all food to the gonimoblast passes through the auxiliary cell which becomes large and nutritive but does not fuse with the surrounding cells. In *Agardhiella*, *Solieria* and *Meristotheca* there is developed, after fertilization, a nutritive tissue of small cells around the auxiliary cell and the growing gonimoblast enters into direct connection with this tissue by special filaments. In *Cystoclonium* the auxiliary cell is of importance as a nutritive cell only during first stages of development of the gonimoblast. After fertilization fusion occurs between the auxiliary cell and surrounding vegetative cells. There is thus formed a large food-containing fusion cell through which food is conducted to the growing gonimoblast. In *Calliblepharis* as in *Hypnea* the auxiliary cell is of nutritive importance only during early stages of development of the gonimoblast. Later on the gonimoblast receives all food from special nutritive tissue which is developed after fertilization. In *Stenogramme*, *Phyllophora* and *Gigartina* the auxiliary cell is already large and nutritive before fertilization but its nutritive importance is limited, however, to the very first stages of development of the gonimoblast. In *Stenogramme* and *Phyllophora* the gonimoblast receives its food from nutritive tissue which is developed before fertilization occurs but in *Gigartina* a special tissue for the purpose of supplying the gonimoblast with food is developed after fertilization.

*Rhodymeniales*. In *Fauchea*, *Rhodymenia*, *Lomentaria* and *Chylocladia* the auxiliary cells, before fertilization, are small and similar to the surrounding vegetative cells and contain either just as much or only a little more food than the latter. But after fertilization they grow considerably and become filled with nutritive material. All food to the gonimoblast passes through the auxiliary cell. A fusion between the auxiliary cell and surrounding vegetative cells occurs sometimes.

*Ceramiales*. The auxiliary cell is here cut off after fertilization and is rich in food already from the beginning. The supporting cell, and often the central cell too, contains a large quantity of food. In addition there are present, as a rule, several very nutritious so-called sterile cells. A fusion occurs between the auxiliary cell, the supporting cell, and the first gonimoblast cell in an early stage of development of the gonimoblast. The fusion cell, thus formed, may later also fuse with other cells. All food to the gonimoblast is conducted through the fusion cell.

It has already been mentioned that a Floridean system which so far as possible has to show phylogenetical development has to be founded primarily on the auxiliary cells and the nutritive tissues. An outline of such a system is presented by Kylin (17). This system is based on statements of previous authors, on his own investigations and on papers by Sjöstedt and Bliding. After the above-mentioned outline of a Floridean system had been presented there appeared a paper by Rosenberg (19) in which he points out that the family Dasyaceae forms an independent group in the order Ceramiales. The Dasyaceae were formerly classified as belonging to the family Rhodomelaceae. In a paper which appeared in 1934, Kylin has shown that in the Rhodomelaceae the carpogonial branch is cut off later than the lateral group of the so-called sterile cells. Previously the carpogonial branch was always considered to be cut off before the sterile cells. The observations of Kylin show that, viewed from the history of development, the four families Ceramiaceae, Delesseriaceae, Dasyaceae and Rhodomelaceae are all closely related to each other.

Concerning alternation of generations in the Florideae two types are distinguished by Svedelius (24): the haplobiontic type and the diplobiontic type. In the latter type, there is in the life-history a regular alternation between individuals bearing gonimoblasts and



those bearing tetraspores. In the former (haplobiontic) type special individuals bearing tetraspores are lacking. In the haplobiontic Florideae reduction-division occurs at the first division of the fertilized nucleus (usually in the carpogonium), but in the diplobiontic forms it takes place, on the contrary, at formation of the tetraspores. Investigations of recent years have, however, revealed some exceptions of great interest to this general rule.

The haplobiontic Florideae belong to the order Nemalionales. To this order also belongs the genus *Liagora*. Börgesen (2) discovered, however, that in one species of this genus, *L. tetrasporifera*, the gonimoblast develops tetraspores instead of carpospores (monospores). Later Kylin (15) verified this statement. It is not known where reduction division takes place in *Liagora tetrasporifera* but it is not at all improbable that it is removed from the carpogonium to the tetrasporangia and in that case the gonimoblast would be diploid, not haploid as in the haplobiontic Florideae.

It has already been known for a long time that *Phyllophora Brodiaei* bears small globular bodies which produce tetraspores. These bodies have been supposed to belong to a parasitic Florideae, called *Actinococcus subcutaneus*. No generative organs were, however, ever observed in *Phyllophora* and some investigators thus declared *Actinococcus* to be nothing but the generative organs of *Phyllophora*. The problem was solved recently by Rosenvinge (20) since he was able to show that *Actinococcus* originates from the auxiliary cell of *Phyllophora*. In *Phyllophora* only sexual individuals are to be found. The auxiliary cells which ought to grow out to gonimoblasts with carpospores (monospores) give rise instead to small globules which produce tetraspores. It could be expressed as follows: formation of carpospores is replaced by formation of tetraspores. According to Claussen (7) reduction division occurs at formation of tetraspores. In *Phyllophora Brodiaei* there are no independent tetrasporic plants. From a systematic point of view *Phyllophora* belongs to the diplobiontic Florideae but in its life-history it is, however, a haplobiontic Florideae, very comparable to *Liagora tetrasporifera*. In another species, *Ph. membranifolia*, there are independent tetrasporic plants as well as plants with gonimoblasts. *Ph. membranifolia* is a typical diplobiontic Florideae. The statement of Rosenvinge concerning *Ph. Brodiaei* has been verified by Kylin (15).



With respect to alternation of generations, the conditions in *Phyllophora* have parallels in the genus *Gymnogongrus*. There are, for example, in *G. norvegicus* both individuals with tetraspores and individuals with gonimoblasts. *G. norvegicus* corresponds to *Phyllophora membranifolia*. In *Gymnogongrus Griffithsiae* only tetrasporic individuals occur. The tetraspores are here produced in small globular bodies which formerly were considered as an independent species, *Actinococcus aggregatus*. Quite recently, Chemin (6) and Gregory (9) stated that *G. Griffithsiae* is a sexual plant and that *Actinococcus aggregatus* is developed from auxiliary cells of *Gymnogongrus Griffithsiae* and that it represents the tetrasporic generation of this alga. *G. Griffithsiae* is a parallel to *Phyllophora Brodiaei*.

The small globular bodies on *Ahnfeltia plicata* were formerly regarded as being a parasitic red alga, *Sterrocolax decipiens*. The globular bodies produce monospores as reproductive organs. Formerly no reproductive organs were known to occur in *Ahnfeltia*. Now, however, Chemin (5), Rosenvinge (21) and Gregory (9) have all been able to show that *Sterrocolax decipiens* represents the reproductive organs of *Ahnfeltia plicata*. It seems, however, that sexual reproduction is lacking in *Ahnfeltia* and that *Sterrocolax* in an asexual manner grows out from *Ahnfeltia*. The fact that *Sterrocolax* gives rise only to monospores and not to tetraspores is obviously connected with the fact that there is no sexual reproduction in *Ahnfeltia*. Individuals of *Ahnfeltia* which bear the *Sterrocolax*-globules represent female individuals. Special male individuals with spermatangia are found also in *Ahnfeltia*.

In the diplobiontic red algae there is regular alternation between haploid sexual individuals and diploid asexual ones. Haploid individuals are bearers of gonimoblasts, diploid ones of tetraspores. Sometimes, however, gonimoblasts and tetraspores occur on the same individuals. The best known species in which this condition is found is *Spermothamnion Turneri* (Sp. *roseolum*). This alga has recently been studied by Kathleen Drew (8) with special reference to cytological facts. From results of her studies the following facts are to be noted. All individuals found growing in the sea were diploid with 60 as the number of chromosomes. Those individuals bore tetraspores as well as fully developed gonimoblasts. In formation of tetraspores reduction divisions took place. The

nuclei of the developing gonimoblast showed it to be tetraploid. Whether the tetraploid carpospores are capable of germination or not was not investigated. When cultivated, the haploid tetraspores germinated into haploid filaments which, however, died when they were three or four cells in length. The statement that the developing gonimoblast is tetraploid is of special interest. It is evident, however, that further investigations are necessary before the problem of the alternation of generations in *Spermothamnion* can be solved.

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## GLOSSARY

The Rhodophyceae, or Red Algae, are known also as the Florideae; this name, as used by some authors, however, excludes one order, the Bangiales. The great majority of red algae are marine; only a few inhabit fresh waters. Their sexual reproduction is quite unlike that of any other algae and has necessitated special terminology, use of which varies somewhat among different writers. In general, however, the following brief discussion will serve as a basis for the accompanying article.

The male organs, *antheridia*, produce non-motile *spermata*. The female organ, *carpogonium*, is borne on the *carpogonial branch* and has an elongated receptive *trichogyne*. The *egg* is at the base of the *carpogonium* and after fertilization does not develop into a new plant but produces *carpospores* in a variety of ways. These spores develop directly from the zygote, or from *sporogenous filaments* which arise from the zygote and constitute the *gonimoblast*. They may also develop from other cells of the thallus into which the zygote nucleus or one of its descendants has migrated. The entire spore-producing structure is known as a *cystocarp*. The *hypogynous cell* is the one immediately below the *carpogonium*.—Editors.

# THE BOTANICAL REVIEW

VOL. I

MAY, 1935

No. 5

## REPRODUCTION AND LIFE HISTORY IN DIATOMS

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(Translated from the German by the editors)

Diatoms (Bacillariales) are unicellular algae with brown chromatophores. They are distinguished from all other algae by their cell-structure, mode of division and life history. The cell-wall is composed of two halves which fit one in the other like the top and bottom of a pill-box. Each half, in turn, is composed of two parts, the *valve*, which corresponds to the top or the bottom of the pill-box, and the *girdle band*, which corresponds to the side-wall. The valves are constructed radially, as in the Centrales, or they possess zygomorphic structure, as in the Pennales. During cell division the two halves separate from one another and each daughter cell produces a new half which fits inside the old one as the pill-box does within its cover. Since the valves are silicified and therefore neither extensible nor capable of further growth, the descendants which arise in each successive division are smaller and smaller.<sup>1</sup> This progressive diminution of the cells is counteracted, however, by occasional formation of particular cells, known as auxospores, which are capable of further growth. The essential nature of their formation is that a cell discards its rigid silicified membrane, thus liberating the protoplast which then grows to the size characteristic of the particular species. After this growth the auxospore again forms two silicified half-membranes. In many cases—and these are probably phylogenetically primitive ones—formation of auxospores is associated with a sexual act, insofar as the cell which bursts the membrane forms gametes or gamete-nuclei which fuse and produce a zygote; the zygote then begins to grow and becomes an auxospore.

In the Centrales there are, in addition, so-called microspores. These are small ciliated (possibly bi-ciliated) motile cells which are

<sup>1</sup> The general morphology of the diatoms has been summarized by Hustedt and by G. M. Smith (12, 21).

produced in numbers within the mother cell and then extruded. Their further history is unknown and it is uncertain, consequently, whether they are zoospores or gametes. The Pennales, on the other hand, do not have ciliated cells.

During recent years the propagation and life history of the Diatoms have been considered in several morphological, cytological and experimental researches. The alternation of haploid and diploid nuclei, which occurs among diatoms as well as in all other sexually reproducing organisms, was investigated at the same time. Since the Pennales and Centrales do not behave exactly alike, they may be separately considered in the following discussion, the Pennales first because they have been the more thoroughly studied. The general conceptions of cell diminution among the Pennales hold equally well for the Centrales.

#### PENNALES

Auxospores of the Pennales arise almost always from zygotes, that is, as the result of a sexual act. Vegetative development of auxospores represents loss of sexuality involving parthenogenesis or apogamy. Microspores and ciliated cells in general do not exist in this group.

The problem of cell diminution and, in connection with it, of the inducement of sexual reproduction or auxospore formation has recently been investigated, involving pure cultures and continued observations in nature on thirty species and varieties (9). Three categories of cells may be distinguished in each species. The first includes *cells of maximum size* which are capable only of cell division and of producing smaller progeny which in turn behave in a similar manner. A certain number of these divisions results in the second class, comprising *cells of much smaller size* which are capable of forming gametes as well as of cell division; these cells can produce auxospores. In species lacking sexual reproduction, auxospores arise from cells of this second size without preliminary gamete formation. Cells of the second class which, because of external conditions, do not produce auxospores, divide further and produce smaller and smaller progeny. These particularly small cells constitute the third category of *very small cells* which no longer are capable of reproducing sexually or of forming auxospores. They divide vegetatively under various depression phe-

nomena until finally their lowest vitality is reached and in all cases, even under the most favorable culture conditions, death ensues. The reason for this lies in the fact that, because of their smaller size, definite physical factors (relative surface, capillarity, surface tension) operate otherwise than in conformity with the organization of the species; not the least of such factors is the approach of the cell components to the molecular size.

Three principal phases of the life history may then be recognized: 1. the beginning of the life history with cells of maximum size; 2. the appearance of sexual or asexual auxospore formation in cells of smaller size; 3. the formation of still smaller cells which finally die and take no further part in the life cycle.

Only in cells of the second category, not in the larger or smaller ones of the first and third classes, can sexual reproduction or auxospore formation be secured experimentally. The essential requirement for its appearance is, under all circumstances, the cell size and correlated internal physiological conditions. In many species the necessary stimulus may be applied by transfer to a medium of weaker concentration involving change in turgor pressure. In like manner auxospore formation can be induced in *Melosira nummuloides*, one of the Centrales (20). In *Navicula seminulum* and *Gomphonema parvulum* light intensity also influences inception of auxospore formation, apparently through changes in turgor pressure resulting from formation of assimilates.

The effect of cell size, which determines physical-physiological changes within the cells, is generally more important than external conditions in deciding whether vegetative cell division or auxospore formation shall occur. Diatoms offer the most valuable material for the general problem of the relation between cell size and organization because they are favorably adapted to study.<sup>2</sup>

With decreasing cell size, there begin, during the course of cell divisions, characteristic morphological changes of shape and of valve-form. It is generally noted that the progeny of a cell is not

<sup>2</sup> One form, *Eunotia pectinalis* var. *minor*, shows an entirely different life history (9). Its cells possess girdle bands which are elastic and bulging in a characteristic manner, as a result of which the progeny do not necessarily become progressively smaller. In this species, also, auxospore formation was not observed. Incidentally it may be mentioned that some marine diatoms produce unsilicified membranes under certain cultural conditions (1, 2, 9). As the more or less abnormal cell-forms show, depression phenomena are involved in such cases.



merely a smaller geometrical likeness of its ancestor but that the cell occasionally possesses a definite shape which is coordinated with its size. It is most striking that one of the three cell axes (the apical or long axis) is shortened more than the other two and the cells thereby become more compact. These and other changes (reduction in bulging, decrease in the number of markings on the valve per unit of area) are of taxonomic importance. In considering material secured in nature, which includes cells of only one particular size, errors, such as the establishment of new species, arise because of failure to observe all changes in form. Changes associated with variations in size affect not only external morphology of the cells but also extend to the structure of the protoplast, involving, for example, changes in development of chromatophores and position of the nucleus; in short, simplifications are generally observed in connection with decreasing cell size.

In addition to shedding light upon form changes, the past years have brought increased cytological understanding, particularly with respect to changes in haploid-diploid phases of the nucleus. It has been shown, first, in confirmation of the classical investigations of Lauterborn, that the numerous newly investigated species, and this is true also for the Centrales, possess mitoses with a central spindle. The chromosomes, therefore, do not form a plate at metaphase but rather a ring (5, 6, 7, 15, 17, 18, 19). This type of mitosis, regarded as fundamental in animals, is very likely characteristic of all diatoms. A second result of recent investigations is that, in all sexually reproducing species, reduction division takes place during gamete formation; such species are accordingly diploid organisms. The Pennales, therefore, constitute the only group of plants which are definitely diplonts<sup>3</sup> like the Metazoa.<sup>4</sup> Therefore the old claims of Klebahn and Karsten, based upon only three species and partly incomplete, are confirmed upon a broad scale. Cytological details of meiosis have not been worked out, to be sure, in any case with the same clarity as in the Metazoa and Metaphyta. However, the fundamental similarity of meiotic processes which prevails in the

<sup>3</sup> For an excellent discussion of nuclear phases, alternation of generations and terminology among algae, especially the Red Algae, see Svedelius, *Beih. Bot. Centr.* 48: 38-59. 1931.—Editor.

<sup>4</sup> In other *almost* diploid plants as the Fucaceae and the Angiosperms there is, on the other hand, a concealed antithetic alternation of generations, since a haploid generation (gametophyte), even though few-celled, does occur.—Author.



entire realm of living organisms can be established, likewise, among diatoms; that is, meiosis consists of two successive divisions, the heterotypic and the homotypic. Chromosome pairing takes place in the customary manner, progressing through the bouquet<sup>5</sup> stage to diakinesis<sup>6</sup> and finally to numerical reduction. The bouquet appears changed through fixation usually as synizesis.<sup>7</sup>

The best known and, because of their considerable size, the most favorable objects for studying these phenomena are *Cymbella lanceolata* and *C. cistula*.

The new investigations also make it possible to secure a complete picture of fusion processes (Cholnoky, Geitler). The gametes are, as has long been known, not ciliated but possess amoeboid movement. In one incompletely explained case (18) the existence of contractile vacuoles was established. These findings are of special interest since the diatoms, like all algae, are descended phylogenetically from the flagellates in which contractile vacuoles are characteristic.<sup>8</sup>

Fusion is initiated by juxtaposition of vegetative mother cells in which reduction division takes place and gametes are formed. Fusion of gametangia thus occurs. The mother cells of the gametes are often sister cells (paedogamy). Contact of the gamete mother cells is followed by bursting of the silica membrane and formation of a gelatinous envelope or fusion sac within which the gametes meet. The fusion sac is a transformation product of one layer of the membrane, composed of pectin and lying just within the silicified membrane in all vegetative cells (14).

The number of gametes is constant for each species and may be one or two. Pascher saw four naked protoplasts in cells of *Nitzschia* which were perhaps gametes; no fusion was observed, however. Since four cells or nuclei must have arisen almost in every case through the two divisions of meiosis, this condition in *Nitzschia* might easily be explained. In the other species two or three nuclei disappear and only one or two gametes develop. In

<sup>5</sup> The bouquet is that stage during meiosis in certain organisms in which the chromosomes lie in loops with their ends near one part of the wall of the nucleus (Darlington).—Editor.

<sup>6</sup> Diakinesis is the last stage in the prophase of meiosis immediately before the disappearance of the nuclear membrane (Darlington).—Editor.

<sup>7</sup> Synizesis is a contraction of the chromosomes to one side of the nucleus (an artifact) (Darlington).—Editor.

<sup>8</sup> Contractile vacuoles have been observed also in vegetative cells of *Rhizosolenia longiseta* and *Attheya Zachariasii* (13).

*Cocconeis* and *Navicula seminulum*, which form only a single gamete, one of the two daughter nuclei arising from the first (heterotypic) division disappears; the surviving nucleus undergoes the second meiotic division and one of the two daughter nuclei here too disappears; the remaining one develops into the gamete nucleus. While elsewhere supernumerary nuclei are resorbed into the protoplasm, in these species the first nucleus to disappear is abstricted from the cell, thus behaving like a polar body in animal oogenesis. This then represents a rudimentary second division. Formation of only one gamete must, therefore, be regarded as phylogenetic retrogression. Less rudimentary is the production of two gametes in one mother cell. The most primitive case, formation of four gametes, is to be found probably in *Nitzschia*, as noted above.

The gametes are entirely alike in both sexes or only slightly differentiated. In many species, however, they are distinctly different physiologically, since one gamete, the male, is motile and moves toward the other which is non-motile and female. There thus prevails a physiological anisogamy similar to that known in *Spirogyra*. If only one gamete forms in each mother cell, then the males penetrate those mother cells harboring the female gametes and there fuse with the latter. In the formation of two gametes, only one gamete of each cell is motile; the other is non-motile (resting gamete). The entire fusion process then occurs in two stages. A gamete of one cell enters the second cell and fuses with the gamete in it. At the same time, or a little later, the other gamete of the second cell travels in the opposite direction to the first cell and fuses with the gamete remaining there. In species which behave isogamously, the gametes meet halfway between the mother cells.

The physiologically anisogamous behavior involved in the formation of two gametes permits the interpretation that each mother cell forms two gametes of opposite sex, a male motile gamete and a female non-motile gamete. The mother cells themselves may, accordingly, be regarded as hermaphroditic and the entire behavior would, from this viewpoint, correspond to that involved in the conjugation of the Ciliates. So far as we know, sex determination occurs phenotypically in all cases hitherto investigated. A sex chromosome mechanism has not been observed nor can it be expected.

By means of fusion there arise one or two gametes from one

pair of mother cells, according to the number of gametes. Nuclear fusion follows in them. According to observations of Cholnoky (6) the sex nuclei during fusion show chromosomal structures of a prophase nature. The zygotes possess only one membrane which is burst during development of the zygote into an auxospore. Auxospore formation is, therefore, a germination process (Cholnoky, confirmed by Geitler, 1932).

Further growth of the auxospores, which is primarily a matter of expansion, ensues parallel to the direction of fusion in the case of isogamous fusion, whereas in the case of anisogamy it takes place perpendicular to the direction of fusion (Geitler). The two auxospores which arise from one pair of mother cells, therefore, are always parallel to one another but lie at right angles to the long axis of the mother cells in the first case and parallel to it in the second. These and other peculiar and constant position-relationships are dependent upon a definite polarity of the cells and need further investigation.

In the light of new and old investigations, the following review of the types of auxospore formation may be presented. Rare cases of retrogressive and lost sexuality are also considered.

1. Two mother cells each form two gametes which fuse in pairs and produce two zygotes (auxospores.)

- a. The gametes are isogamous.

- b. The gametes are anisogamous.

- c. In some cases it can not be determined whether the gametes are isogamous or anisogamous, since the gelatinous sheath, within which fusion occurs, is very soft and the gametes can move about freely within it (9).

2. Two mother cells each form one gamete, resulting in one zygote (auxospore).

- a. The gametes are isogamous.

- b. The gametes are anisogamous.

3. In a single mother cell one zygote develops through automixis<sup>9</sup> (auxospore).

- a. Two gametes develop in the mother cell which fuse and produce a zygote (auxospore).

- b. Two surviving nuclei unite within the undivided protoplast to form a zygote nucleus; the protoplast then becomes a zygote.

<sup>9</sup> Automixis is self-fertilization following copulation of two closely related sexual cells or sexual nuclei (Gaumann and Dodge).—Editor.

4. Auxospore formation occurs without sexuality.

a. Parthenotically; two mitotic divisions corresponding to meiosis occur which, however, are vegetative divisions (diploid parthenogenesis) as in metazoan eggs.

c. Apogamously; completely lacking all indication of sexuality.

Some special peculiarities may well be included in this review (9). In *Cymbella sumatrensis* vegetative nuclear division without cell division takes place in the auxospore which has arisen according to Type 1b. One of the two daughter nuclei disappears and the second becomes the true nucleus of the auxospore which then develops normally. An accurate interpretation of this process is not yet possible; it may be supposed, however, that a preliminary rudimentary cell-division does take place which is represented by the surviving nuclear division. In this connection it is of interest that the first division of the auxospore in species of *Cocconeis* proceeds irregularly and produces two unequal daughter cells. The one daughter cell has normal structure and continues to develop; the other is always irregular in a definite manner and has no raphe.<sup>10</sup> This second cell is not capable of further normal development. So in this case also only a single normal cell forms after a division.

In conclusion, it may be said that the Pennales possess primary sexual reproduction. Alternation of nuclear phases is apparent because meiosis enters into gamete formation. The Pennales, like the Metazoa, are, therefore, diploid organisms. Auxospore formation occurs simultaneously with zygote production. Many species have lost sexual reproduction and in them auxospores arise parthenotically, or apogamously.

#### CENTRALES

The general life history of the Centrales is, for the most part, the same as described for the Pennales. Here, also, the cells become smaller and smaller during the course of cell divisions and the original size is restored through formation of auxospores.

While in the Pennales the general principles of sexual reproduction and auxospore formation are understood, this is not true for the Centrales. During recent years several investigations have been directed at this problem but without yielding conclusive general

<sup>10</sup> The raphe is a cleft in the membrane through which streaming protoplasm protrudes, causing the well known creeping movements of the cells.

clarification. The present status of the investigations may now be discussed.

The most significant deficiency in our understanding is that the behavior of the microspores has never been exhaustively observed. The old assumption of Karsten, that the microspores fuse with each other isogamously, is unproven, since it is not supported by observation. Schmidt (19), working with *Biddulphia*, and Hofker (10) with *Coscinodiscus*, recently advocate the same idea, but without having observed fusion of the microspores. The opinion of these authors is founded on the fact that divisions take place during formation of microspores which might be regarded as constituting meiosis. The cytological picture, however, is not so clear as in the Pennales and the evidences of meiosis are not entirely conclusive. It is possible, nevertheless, that the interpretation of Schmidt and Hofker may be correct, in which case the microspores would be isogametes which fuse with one another and the Centrales which have been investigated would be diploid organisms like the Pennales. In contrast with the Pennales, however, formation of zygotes would not be associated with production of auxospores; the auxospores would develop vegetatively instead and at another point in the life history.

Persidsky (17) made entirely different observations in connection with *Chaetoceros*. At the beginning of auxospore formation the nucleus undergoes two divisions; of the four daughter nuclei two disintegrate while the other two fuse within the plasm of the mother cell and become the auxospore nucleus. Persidsky regards the two cell divisions as constituting meiosis. Autogamous fusion, therefore, takes place and the general behavior is the same as in Type 3b of the Pennales. Auxospores would then arise from zygotes and the behavior would agree fundamentally with that of the Pennales. Persidsky regards the microspores as zoospores. The cytological observations are certainly not sufficiently detailed that this idea may be regarded as definitely established in all respects. It is particularly significant that three of the four nuclei are smaller than the fourth, almost conveying the impression that three nuclei disintegrate rather than two. If three nuclei actually do disappear, the ensuing fertilization must be by a gamete from some other source. The following observations are particularly significant in this connection.

In another species of *Chaetoceros*, F. W. Went made some observations which, though incomplete and therefore inconclusive, may perhaps supply the key to an understanding of the entire problem of reproduction among the Centrales.<sup>11</sup> Went observed that cells with undivided contents were surrounded by microspores which had been formed in other cells. Went assumes that this was the initial stage of a fusion; the actual fusion, however, was not seen. If fusion does occur, then the microspores would be regarded as small male gametes (spermatozoids) which escape from the mother cells and swim to the female cell, which in turn contains an undivided female gamete (egg-cell). The contents of the undivided cells would, therefore, be fertilized and the resulting zygotes would develop into auxospores.

If these assumptions are correct, then the contradicting claims of Schmidt and Hofker on one side and of Persidsky on the other may be accounted for. If the microspores are spermatozoids, we can understand why no one has observed fusion among them, although there is meiosis during their division. Schmidt and Hofker may, therefore, have observed only the formation of male gametes; Persidsky, on the other hand, may have investigated female cells in which reduction division likewise occurs, followed by formation of zygotes (auxospores). Nuclear fusion, however, would not occur through autogamy, as Persidsky claims, but would be the fusion of the fourth surviving nucleus with one nucleus of a penetrating microspore. In any case, Persidsky did not observe microspores. It is quite possible, therefore, that his claim for autogamy is correct, all the more so because autogamy does occur in *Melosira*. Different species can behave in different ways, either as described by Went or by Persidsky. Likewise among the Pennales some species of one genus show autogamy (*Achnanthes subsessilis* according to Karsten), while others show allogamy (*A. lanceolata* according to Geitler).

If the above mentioned conception is correct, then a general understanding of all Centrales may be possible, though at present it may be only a working hypothesis. The Centrales appear to be phylogenetically the more primitive group in contrast with the Pennales, since the former exhibit fusion of gametes and the latter

<sup>11</sup> A short article appears in Nederl. Chron. Arch. 1924-25. A letter received by me on this subject is presented on page 11 of the 1932 volume.



fusion of gametangia. The fundamental uniformity of diatoms, which undoubtedly exists in their vegetative structure, is apparent also in their reproduction, since in all cases sexual reproduction and reduction division appear to be associated with auxospore formation.

Of great significance in this connection are the new investigations on *Melosira*. Cholnoky observed in *M. arenaria* that the cells which form auxospores contain three nuclei, one of which is larger than the other two. The two small nuclei disintegrate and the larger one becomes the nucleus of the auxospore. Cholnoky thinks that meiosis occurs before auxospore formation, that two of the four nuclei perish and that the two surviving ones fuse with each other. Autogamy would then prevail, as Persidsky claims, for *Chaetoceros*. Persidsky was able to prove this assumption to be an actual fact in *Melosira varians* without knowing the observations of Cholnoky. In mother cells which later develop into auxospores, he observed two nuclear divisions which represented heterotypic and homotypic divisions of meiosis. The cells, therefore, contain four nuclei, of which two degenerate while the other two fuse with each other and the syncaryon<sup>12</sup> becomes the nucleus of the auxospore. The latter, therefore, arises from an autogamously formed zygote.

It is accordingly certain that auxospores in *Melosira* do not arise purely vegetatively, as other authors have assumed to be a general condition in the Centrales, but that their formation is associated with a sexual act. Microspores are not known in *Melosira* and the problems concerning them, therefore, still remain unsolved. According to the working hypothesis stated above, *Melosira* may be regarded as phylogenetically retrogressive in contrast with other Centrales which possess microspores. In comparison with *Coscinodiscus* and *Biddulphia*, *Melosira* is more closely related to the Pennales. As far as reproduction is concerned, it behaves exactly like group 3b of the Pennales.

#### SUMMARY

The Pennales are diplonts and possess sexual, parthenogenetic or apogamous auxospore formation. Fusion is either isogamous or physiologically anisogamous.

<sup>12</sup> The syncaryon, here, is the fusion product of the two surviving nuclei.—Editor.



Sexual reproduction among the Centrales, with the exception of *Melosira*, is not fully understood. It is probable, however, that the Centrales are also diplonts. Contradicting claims of various authors can be explained through the assumption that the microspores are spermatozoids which fertilize the undivided contents of female cells (egg cells) and that the resulting zygotes develop into auxospores. Conclusive proof of this claim is lacking for neither do cytological investigations of reduction divisions which occur during microspore formation suffice in the matter, nor has fusion of the gametes been observed. In *Melosira*, which has no microspores, reduction division takes place in the mother cells before auxospore formation; two of the four nuclei fuse and the zygote develops into an auxospore.

The problems concerning sexual reproduction and alternation of nuclear phases among Centrales still remain unsolved except for *Melosira*. The Pennales, on the other hand, are definitely diploid.

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# AUXIN, THE PLANT GROWTH-HORMONE

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## I

Over fifty years ago Julius Sachs (48) clearly pointed out that correlations in plants most likely were caused by "specific substances," not of the nature of foodstuffs but directing the activities of cells in extremely small quantities. Twenty-five years later hormones in animals were discovered, substantiating the principal point of Sachs's theory; but it took another twenty-five years before botanists generally became aware of the soundness of his reasoning. It is clear now that plants furnish most favorable material to study "correlation carriers" or phyto-hormones. The following report on the plant growth-hormone or growth-substance (g. s.) substantiates this statement. The physiological name *growth-substance* and the chemical name *auxin* are interchangeable.

Of the different growth stages (initiation, differentiation, elongation and maturation) elongation is the most spectacular and the one that can best be measured since it involves the greatest change in dimensions. It is in this stage of cell elongation that the more or less embryonic cells of organ primordia enlarge to their final size. This elongation is, in most cases, accompanied by some cell divisions, which, however, are of minor importance. In *Avena* coleoptile,<sup>1</sup> which is the main experimental plant in investigations on g. s., no cell divisions occur in the stages in which it is used (61) and any factor affecting growth influences cell elongation only.

Now growth in length furnishes one of the finest examples of correlations in plants. For a long time it has been known that most stems, petioles, flowerstalks and coleoptiles stop growing if the apical, only slightly growing parts (end buds or leaf blades, flowers, or tips, respectively), are cut off. Paál (46) was the first to demonstrate that this is not due to a simple wound shock, for, if the tip of a decapitated coleoptile is replaced on the cut surface, the

<sup>1</sup> The coleoptile is a leaf-sheaf which envelopes the growing point and first foliage leaf.—Editor.

stump will grow faster than without the tip (Fig. 1: demonstration) (52). It appears that this influence of the tip is caused by some substance diffusing out of it, as a layer of gelatin will not impede this action. For many other plants and organs it can be shown (53, 73) that replacement of the cut off apical parts at least partially restores the original growth rate.

## II

As a consequence of this and other work Went (75) demonstrated that the growth promoting factor from coleoptile tips will diffuse out of them into a layer of agar or gelatin if they have been standing on it for some time (Fig. 1: collection). A set of decapitated coleoptiles will grow considerably faster if blocks of this agar are placed on their cut surfaces than if they are supplied with pure agar (Fig. 1: demonstration). This principle of influencing growth with the exudate of tips, diffusing out of agar blocks, has been worked out as a quantitative method of determining the amount of growth-substance dissolved in agar (75, 78). To rule out more or less individual variations each determination of g. s. is made with 12 plants. They are raised under exactly constant conditions: darkness (only red or orange light is used to carry out the manipulations), constant temperature of 20° or 25° C., constant humidity of 90 per cent and absence of traces of toxic gases. When the plants have reached a length of 30–40 mm. they are decapitated and their first leaf (inside the coleoptile) is pulled loose to prevent it from growing out and pushing the agar block off the cut surface (Fig. 1: detection). An agar layer, 8 x 6 x 1 mm., containing auxin is cut into 12 equal pieces, 2 x 2 x 1 mm., so that each little block carries the same amount of g. s. (Fig. 1: collection). Forty minutes after decapitation such an agar block is stuck on one side of the cut surface of each of the 12 plants (Fig. 1: detection). As the transport of the hormone is strictly longitudinal, only the side under the agar block will have an extra supply of g. s. and, consequently, will grow faster than the opposite side. This results in a curvature away from the agar block, which is measured about 2 hours after application of the agar (Fig. 1: detection). Over a range of about 1° to 20°, curvatures are strictly proportional to concentration of g. s. in the agar (Fig. 1, lower right; 65). Thus, the mean curvature of 12 plants provides an accurate

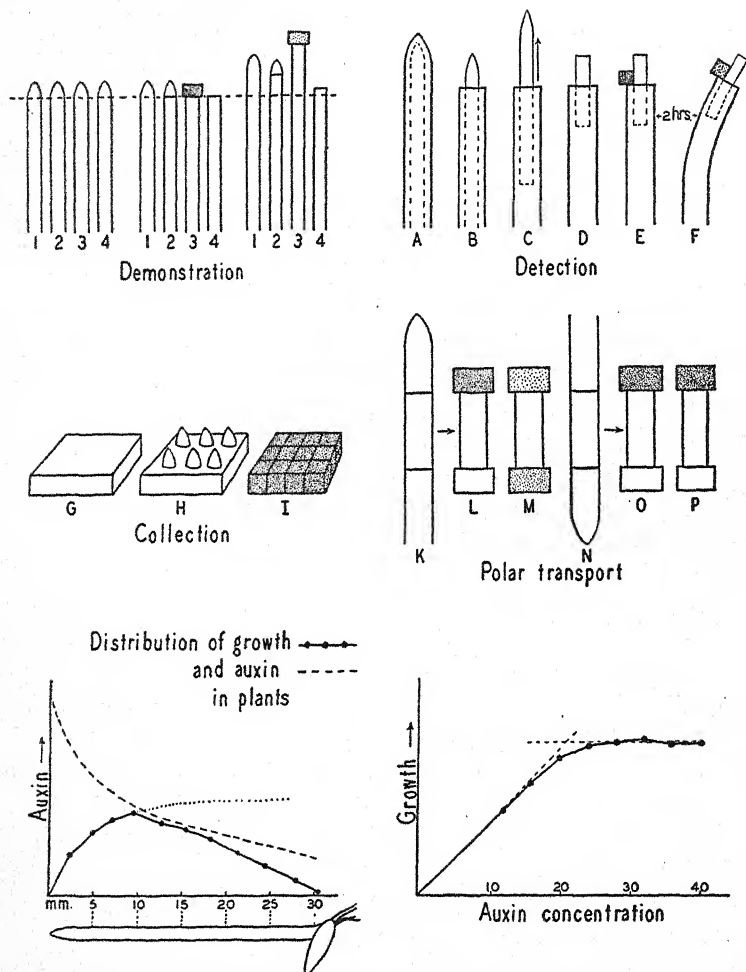


FIG. 1. Auxin (its concentration in the agar is shown by various shades of dotting) and growth of *Avena* coleoptiles.

*Upper left.* Demonstration of auxin formation by coleoptile tip. Plant No. 1 is left intact; Nos. 2, 3 and 4 are decapitated. On No. 2 the cut tip is replaced; on No. 3 auxin (in agar) is stuck. The right hand set of four plants shows the effect of this treatment on growth after three hours.

*Middle left.* Collection of auxin from cut coleoptile tips. For a period of 2 hours 6 coleoptile tips are placed (H) on a layer of agar,  $6 \times 8 \times 1$  mm. (G). After removal of the tips the agar contains auxin diffused out of the tips, and is cut into 12 blocks (I).

*Upper right.* Scheme or test method for auxin. Coleoptile (A) is decap-

determination of the concentration. If blocks of agar have been soaked for an hour in some solution, its g. s. content can be calculated, as auxin easily diffuses into the agar. This content is expressed in arbitrary g. s. units which are reproducible under exactly defined experimental conditions. Kögl and co-workers accept the A. E. (*Avena* Einheit) which is the amount of auxin present in one block of agar,  $2 \times 2 \times .5$  mm., causing a curvature of  $10^\circ$ . The auxin unit of the Kerckhoff Laboratories, Pasadena, expresses the amount of g. s. present in 1 cc. of solution and equals 80 A. E. A plant unit is the amount actually applied in agar and is  $1/200$  of this amount. The *d*-Werte of Nielsen (43) have not been compared directly with the above units. For qualitative work auxin can be mixed with lanolin (39) which prevents the desiccation inherent in working with agar blocks in a dry atmosphere. Recently a new and simpler test method for g. s. has been described (77) using pea seedlings as experimental plants which are placed directly in the solutions to be tested.

### III

With the *Avena* test the chemistry of growth-substance has been worked out especially by Kögl, Haagen Smit and Erxleben (29,

itated leaving the primary leaflet (B). The latter is partly pulled out (C and D) and an agar block with auxin is placed on one side of the cut surface of the coleoptile (E). Two hours after application of the agar the resulting curvature (F) is measured.

*Middle right.* Demonstration of polar transport of auxin. On the apical surface of a coleoptile cylinder cut from the seedling (K) a block of agar with known auxin concentration is placed and the basal surface is placed on a block of pure agar (L). A few hours later the greater part of the auxin will have been transported towards the lower block (M). If the coleoptile cylinder has been reversed (N and O) no transport whatsoever is detectable; all auxin remains in the upper block, in contact with basal cut surface (P).

*Lower left.* On the abscissa zones of a coleoptile (in mm. from the tip) are marked; on the ordinate growth rate (corresponding with drawn line) and auxin concentration (corresponding with broken line). From 9 mm. downwards growth rate is determined by auxin concentration (with allowance for "aging" of zones more than 20 mm. from tip). In the upper 9 mm. a hypothetical factor (extended as dotted line) limits growth rate.

*Lower right.* Relation between applied auxin concentration (abscissa) and rate of curvature or growth (ordinate). Below a certain point (marked 20) growth rate is directly proportional to auxin concentration; above this point a second internal factor starts to limit curvature or growth rate.

35). Also Nielsen (43), Dolk and Thimann (17), and Thimann (63) contributed valuable work along this line. Kögl and co-workers have isolated three different crystalline substances, all giving positive reaction in the *Avena* and pea test, and, therefore, being growth-substances; physiologically they can not be distinguished. They have been named auxin *a* ( $C_{18}H_{32}O_5$ ); auxin *b* ( $C_{18}H_{30}O_4$ );<sup>2</sup> and hetero-auxin ( $\beta$ -indolyl-acetic acid,  $C_{10}H_9O_2N$ ); and all three of them are monobasic acids of about the same strength and have one double bond. Auxin *a* forms a lacton of the same empiric formula as auxin *b*, which is also active as growth-substance.

Auxin *a* and *b* are heat- and light-stable but are easily oxydized; the crystals lose their activity in a few months by isomerisation. They are readily oxydized by oxydase from the plant cells; this explains why ordinary water extracts of plant tissues do not have any effect upon growth (62). The activity of all three auxins is about the same and extremely high, of the order of  $5 \times 10^7$  A. E. per mg. The *Avena* test thus is one of the most sensitive micro-chemical tests. No other pure substances have been found to have any direct effect on growth at all; if saliva, diastase or other products have a growth accelerating effect, this is due to traces of one of the auxins.

One of the richest sources for auxin preparation, human or mammalian urine, contains from 1000 to 5000 A. E. auxin per mg. From malt as well as from corn-germ-oil both auxin *a* and auxin *b* can be prepared (32). Micro-organisms produce auxin (apparently hetero-auxin (33, 63)) in large quantities, synthesizing it from the culture solution (43, 29, 67, 7). Pollen and seeds are the richest higher plant sources of auxins (38, 69).

#### IV

The three auxins are physiologically indistinguishable, all of them giving the same type of growth response. Because they are analyzed by physiological tests it is very difficult to say anything general about the distribution of auxin *a* and *b* and hetero-auxin in relation to the natural system of the plant kingdom. The crystals of auxin, of course, are recognisable by their melting point,

<sup>2</sup> Auxin *b* is in no way related to *B*-Wuchsstoff of Nielsen; it is, like the other auxins, an *A*-Wuchsstoff.



etc., but Kögl, *et al.* (32, 34) succeeded in distinguishing between them even in impure solutions by their different behavior toward acids and bases, and by their diffusion coefficient which allows the approximate calculation of the molecular weight (75).

Auxin *a*: stable in acid, destroyed by alkali; mol. weight 328.

Auxin *b*: destroyed by acid, destroyed by alkali; mol. weight 310.

Hetero-auxin: destroyed by acid, stable in alkali; mol. weight 175.

On account of these properties it was possible to show that in coleoptile tips of corn auxin *a* is formed. From corn-germ-oil as well as from malt crystalline auxin *a* and *b* can both be prepared. So it seems probable that in the higher plants auxin *a* (and perhaps auxin *b*) are formed as *the* growth-substance. In the lower plants Kögl and Kostermans (33) and Thimann (63) could prove that hetero-auxin is formed, and not auxin *a* or *b*, probably from the tryptophane of the culture medium. Rhizopin (43) must be identical with hetero-auxin.

About the distribution of auxin inside a plant almost nothing is known, owing to its inactivation in plant extracts. Only the regions of production are known: end buds, axillary buds (68), leaves (1), cotyledons and coleoptile tips (and perhaps root tips, see paragraph 8). Thimann (62), however, by direct extraction with chloroform could determine the actual concentration of auxin in the *Avena* seedling. It is highest in the tip where it is produced and thence drops towards the base where it is used up in growth.

In most plants the cutting of a g. s. production center means irreversible loss of the capacity of auxin formation. In coleoptiles of grasses, however, all cells potentially possess this capacity. Some hours after the tip has been cut off the cells nearest the cut surface will start to produce auxin again and growth of the stump is resumed. This regeneration of the physiological tip depends upon a number of circumstances such as temperature (70), place of decapitation (82), presence of a free cut surface (60). It is significant for the importance of auxin production in the plant that the sensitivity for stimuli is almost lost during the period between cutting of the tip and regeneration of the physiological tip, but is restored immediately afterwards (15). Perry (47) has described cytological changes in the cell connected with auxin production.

In some organs no center of g. s. production has been found but

it seems to be present everywhere; in such plants decapitation does not have any influence on growth rate. *Lupinus* hypocotyls belong to this type (14). However, if the central cylinder containing the vascular bundles has been cut out, the cortex tissues considerably decrease their growth rate which is restored by addition of growth-substance (12). In this case, the phloem seems to contain or produce the auxin. It is assumed that the formation of g. s. in coleoptile tips involves the transformation of a precursor—in itself inactive—into the physiologically active form. No direct evidence has been brought forward to support this view but many phenomena like regeneration can be explained by it. Another explanation of this regeneration (68) assumes that formation of auxin occurs in the lower zones of growing organs only if their auxin content falls below a certain value; the authors conclude no regeneration can take place there as long as formation in the tip goes on. After decapitation the g. s. concentration decreases resulting in the possibility of regeneration in the lower cells.

We are but poorly informed about the effect of light on auxin production. It drops in prolonged darkness; so, in the long run, light is necessary (45). Only in seedlings is g. s. formed in the dark, apparently from reserves in the seed. In *Avena* coleoptile it is also known that light has a direct effect on diffusion of g. s. from the tip into agar; this phenomenon explains the "long" light-growth reaction (75, 9). In *Raphanus* cotyledons no such effect was found (45).

## V

Experimental work, especially by van der Wey (79, 81), on transport of auxin in the plant has produced results which are remarkable not only in themselves but also from a general standpoint. Auxin is the only substance, occurring naturally in the plant, whose translocation can be followed directly, as almost 100 per cent of the administered growth-substance can be recovered or accounted for by the *Avena* test. The experiments are carried out by placing two blocks of agar, one with and one without auxin, on either a cut surface of a coleoptile cylinder or other tissue to be investigated (Fig. 1: polar transport). If the original concentration of g. s. in the agar is known and the final one (e.g., after an hour's transport) is determined experi-

mentally, the transport can be calculated. In the first place it is found that rate of transport exceeds rate of diffusion by many times. Now we must distinguish between velocity and capacity of transport. The velocity, about 10 mm. per hour, is hardly influenced by temperature and not at all by the length of the tissue through which it passes, nor by the concentration gradient. The capacity of the transport (the amount of auxin transported per unit of time) depends on the concentration gradient but not on the length of the path of transport; whether g. s. has to be transported through a 2 or 6 mm. cylinder does not change the amount transmitted. The effect of temperature on capacity (in contrast with velocity) of transport is very marked; under 40° it is a typical optimal curve with a sharp optimum at 30°–35°; above 40° a completely unexplained second optimum occurs at about 50°. All these facts (except the last) point to the conclusion that transport of g. s. inside the plant is intimately connected with life processes and is not explainable by physical forces alone. In agreement with this conclusion is the fact that narcotization with ether stops transport beyond diffusion rate completely. This inhibition is reversible within certain limits of ether concentration. In the lower ones only the capacity of transport is decreased, the velocity remaining normal (81).

Bottelier (6) recently investigated protoplasmic streaming in *Avena* coleoptile in its dependance on temperature, light, gravity, etc. The velocity of streaming (about 30 mm. per hour) is about constant between 17° and 35°, at least in young coleoptiles which are used for g. s. experiments. Intensity of streaming (*i.e.*, the amount of protoplasm in actual rotation), however, increases with temperature up to an optimum at about 30°.

There is a close parallelism between auxin transport and protoplasmic streaming, and as transport is too fast to be explained by diffusion, the old theory of de Vries seems to explain the facts: namely, auxin is transported inside the cell by protoplasmic streaming which is about 3–4 times as fast as the observed auxin transport velocity. Resistance in transport has to be sought, then, in diffusion of g. s. from one cell to the next, for it has been found that in the coleoptile transport of g. s. takes place through long parenchyma cells and not primarily through the vascular bundle.

A second group of phenomena is connected with direction of g. s.

transport. Auxin is transported only basipetally, *i.e.*, a transport against polarity towards the tip does not take place (75, 79, 81, 45, 37). This polarity also reversibly disappears upon narcotization (81). A still more remarkable demonstration of polarity in growth-substance transport lies in the fact that it is transported against a gradient: an auxin concentration at the base of a coleoptile cylinder of 10–20 times the concentration at the apical end does not impede transport in a basal direction (79). To cover these facts the theory has been proposed that naturally occurring potential difference in the coleoptile and other organs (apical parts being negative against basal parts) would produce an electrophoresis of the negatively charged ions of auxin towards the positive base (76, 28). This is, however, not the place to discuss this theory; it is only mentioned because tropistic curvatures are generally explained now on an electrical basis.

## VI

The properties, formation and transport of auxin having been described, using the *Avena* test as a means of investigation and not as a problem in itself, we have next to consider what part g. s. plays in causing curvature, *i.e.*, in growth. It has been found, at least in *Avena* coleoptiles, that auxin is an indispensable prerequisite for growth in length; without growth-substance there is no growth (75, 16). If an *Avena* coleoptile is decapitated growth rate drops steadily (because the auxin still present in the tissues after decapitation is gradually used up), until regeneration of the physiological tip. If the latter is cut off just before it starts to form g. s., regeneration is delayed for another few hours and growth mostly completely stops. During this period of arrest growth can be restored immediately at any moment by application of auxin. In this case growth is directly proportional to the amount of auxin applied and even can be increased above normal. But soon a limit is reached above which increase of applied auxin concentration does not increase growth any further (Fig. 1: right hand graph) (66, 14). This means that g.s. is no longer the factor limiting growth but one of the other factors, on which the complicated process of growth depends, begins to limit it; from that moment on, auxin is present in excess. This certainly is one of the finest examples of Blackman's theory of limiting factors for there is only a very short

zone of transition where auxin together with the other factor influences growth rate. These facts together with results of decapitation experiments enable us to draw the conclusion that in the normal plant it must be mainly auxin which determines growth rate; only if growth is increased beyond normal do other factors become limiting. (Demonstration of this is furnished by the left hand graph on Fig. 1, where growth rate of a coleoptile appears to be determined, at least in the lower zones, by its auxin content.) The same limitation is met in experiments with unilateral application of g. s.; over a certain range curvatures are directly proportional to concentration but above that they can not be increased (Fig. 1: right hand graph). Then some other factor limits rate of curvature (75, 43, 78) and it is likely that this is not the same factor as in straight growth experiments (10).

Curiously enough our knowledge of this second growth factor is restricted to suppositions. It may be that it is a second hormone, transported upward from the seed or roots, or it may be identical with "cell stretching materials" (75) or perhaps "aging" of the cells has something to do with it (9). As "aging" we have to understand the fact that old cells hardly react at all with auxin. By experimentally stopping growth for some time (by decapitation) cells will be aged and their growth rate will irreversibly be decreased. That lower zones of a young organ gradually stop growth must be explained, then, not only by decrease in auxin concentration (75) which falls towards the base, but also by decreased reactivity of the "aged" cells further from the tip (Fig. 1: left hand graph 1). Still, in some cases at least, growth may stop because all g. s. has been used up in more apical parts. In those cases, growth will be resumed (though slowly, because the cells "aged" during the arrest of growth) upon auxin application (75).

## VII

The question of the mechanism of auxin action has given rise to a considerable number of papers by Heyn, Söding, Bonner, and Strüger. The main result of their investigations is that auxin reacts with the protoplasm of a cell only as the last link in the reaction chain: g. s.—growth, the cell wall properties are changed, causing their elongation. Intermediate stages are still completely unknown.

Growth in length of a cell might be induced either by increase of the force stretching the cell wall, that means by osmotic pressure, or by change in mechanical properties of the cell wall. From previous work, especially by Ursprung and Blum (72), it is clear that osmotic pressure of the cell sap does not increase in growing cells; on the contrary, it may slightly decrease, whereas suction force increases. From this fact the conclusion can be drawn that in growing cells, in the first place, extensibility of the wall changes. Though turgor is a formal necessity for growth it is not its cause.

It has been possible to show (23, 26, 27, 54) that it is primarily plasticity of the cell wall which changes during growth. Direct proof has been given that this plasticity is increased by auxin. This means that the cell wall can be stretched irreversibly by turgor. At the same time elastic extensibility may or may not increase; these changes in elasticity are correlated with growth but not with g. s. Increased elasticity is a result of growth, increased plasticity its cause. That these two properties of the cell wall change independently (another instance is that at 0° C. no changes in elasticity occur, whereas plasticity increases upon g. s. supply) has been explained by a lamellar structure of the cell wall (24). An investigation has been started by means of X-rays to study the effect of auxin on the molecular structure of the cell wall (25).

Lately, Söding (56) has assumed that the primary cause of growth lies in the active intussusception of new material in the cell wall. However, at 0° C. growth caused by auxin takes place normally whereas intussusception is stopped (5). Also from other experiments it is clear that intussusception and growth are two independent phenomena. If the latter is stopped by lack of auxin, the former may go on, making the cell wall more rigid and less stretchable. In other words, this is "aging" of the cells (9). In normally growing cells increase in substance, *i.e.*, in rigidity, by intussusception is kept in balance by plastic stretching. We can conclude these considerations with the paradox that growth of the cell wall, as measured by increase in solid matter, is opposed to the actual growth process we are studying, namely, growth in length by auxin. In this respect an interesting article on growth of the seta of *Pellia* (hepatic) of Overbeck (44) may be mentioned. It brings a number of new observations relating to the problem of growth as passive stretching only or as active intussusception.



Bonner has investigated stages of auxin action preceding change in plasticity. Aerobic metabolism must take place to induce the latter for growth can be inhibited by the same concentrations of KCN and phenyl-urethane which inhibit respiration. Also in a nitrogen atmosphere no growth takes place. On the other hand, there is no appreciable increase in respiration upon application of auxin (the effect of crude g. s. preparations on respiration is due to impurities), so that the part of respiration necessary for g. s. action must be extremely small. The conclusion is reached that respiration is a formal prerequisite for growth; without respiration there is no auxin action.

In several papers Strugger (57-59) demonstrated that growth can be induced also by application of acids. The acids were supposed to act directly on the protoplasm, shortcutting the stages of auxin action by increasing its imbibition pressure in general, resulting in increased growth. At the iso-electric<sup>3</sup> point of the protoplasm (pH = 5.1) hydration would be lowest and growth would fall to a minimum. In this general theory of growth many facts are disregarded, especially the very specific action of auxin on growth. Bonner (4) was able to show, at least in *Avena* coleoptile, that the effect of acid on growth has nothing to do with physical properties of protoplasm but can be explained quantitatively by the setting free of a certain amount of undissociated auxenolic acid, the chemical form in which auxin is active. In contrast to Strugger he did not find a double growth optimum in different acidities. The simple optimum in acid surroundings is caused by change in acidity of cell contents produced by application of acid buffers; this activates most of the auxin still present in the cell at once. No growth stimulation by acid beyond the amount corresponding with the auxin originally present in the cell has been observed. The figures of Gessner (20) on plasticity of cell walls in different pH<sup>4</sup> suggest that the same explanation holds for *Helianthus*, Strugger's object.

### VIII

In the preceding discussion roots have not been mentioned because of their exceptional behavior to auxin. Their growth is

<sup>3</sup> The iso-electric point is that degree of acidity or alkalinity of a medium at which a substance behaves neither as a base nor an acid.—Editor.

<sup>4</sup> pH values indicate degrees of acidity and alkalinity; 7 is neutral, higher values are alkaline, lower values acid.—Editor.



almost completely limited to the extreme tip. Growth in length is a mixture of cell division and cell elongation which are going on at the same time.

Also, in roots, decapitation has an effect on growth. If only the extreme tip has been cut off, a correlation effect causes an increase of growth rate (12). As in the coleoptile, replacement of the tip more or less restores normal growth; this means it decreases growth rate. It was obvious to suppose that here also auxin was producing the effect, especially as the coleoptile tip exerted the same influence as the root tip. Indeed, Nielsen (43) and Boysen Jensen (8) could show that growth of roots stops completely if they are placed in an auxin solution. The same experiment was repeated by Kögl (34) with crystalline auxin with the same result. If applied to one side of the growing zone of the root, curvatures towards the g. s. appear, also indicating a decrease in growth rate (39).

For some time the source of g. s. in roots was unknown because all experiments in extracting auxin from root tips by placing them on agar failed (21). Boysen Jensen (8), however, succeeded by adding 10 per cent glucose to the agar. If root tips are put on this glucose-agar for some time the latter will contain g. s.. The explanation for this result is that the tips do not contain enough reserve food materials to produce auxin by themselves but with the help of glucose they can. Lately Thimann (62) could confirm the exosmosis of g. s. from root-tips placed on glucose-agar but he never obtained quite as much auxin from them by this method as by extracting with chloroform. Coleoptile tips, on the other hand, continue to produce g. s. for many hours when placed on agar. His conclusion is that auxin only accumulates in the root tip but is not formed there (in accordance with the polarity theory (76)).

So in the case of roots for the first time we find that g. s. acts as an inhibitor of growth. Cholodny (13), however, pointed out that in this case decreased growth in length is accompanied by increase in thickness.

## IX

There are other phenomena which depend on auxin. In most cases definite proof has not been furnished that only auxin is the active principle, so they will be mentioned only briefly. However, in the case of bud-inhibition we are sure that it is produced by

auxin, as Kögl's crystalline auxins produced the effect which was attributed to g. s.

It was established, especially by Dostál (18), that the reason why axillary buds temporarily or forever stay dormant is to be sought in a correlation phenomenon. In his cases inhibition was produced by the leaves. In young bean plants the end bud and young leaves were most active in inhibiting outgrowth of axillary buds, as Snow (50) demonstrated. In this case the correlation effect proved to be complex because a stimulus passing through the xylem as well as one being transported polarly in basal direction only were active. The latter had to be caused by a phyto-hormone as it diffused through a wound gap.

Now Thimann and Skoog (68) were able to replace the inhibiting effect of the end bud by a constant supply of auxin. Axillary buds on the first two nodes of young bean or pea plants never develop unless the end bud is cut off. As soon as this has been done they start to grow. This development, however, is completely checked if in place of the cut end bud, a block of agar with suitable auxin concentration is stuck and changed every six hours to insure a constant supply. Application of an auxin solution is as effective. Whereas the axillary buds of decapitated plants grow out as rapidly as those on similar plants treated with pure agar, they do not develop at all on the decapitated plants treated with a concentration of 1000-5000 units g. s. per cc., nor on normal plants. The concentration of auxin giving a complete inhibition is roughly the same as the amount of auxin produced by the end buds and this fact in connection with the effectiveness of crystalline auxins proves that in the normal plant, outgrowth of axillary buds is inhibited by auxin flowing from the end bud.

Uhrova (71) inhibited growth of axillary buds of *Bryophyllum* with the exudate of leaves containing g. s. Laibach (39) and his students obtained the same effect with pollen g. s. on outgrowth of axillary buds of the cotyledons.

With his method of applying auxin mixed with lanolin a number of other effects of g. s. were noticed by Laibach (39). In all these cases, however, final proof that auxin action is involved has to be given with crystalline auxins. Among these other effects may be mentioned growth of style and ovary in orchid flowers which occurs after pollination. This phenomenon was studied first by

Fitting (19) and ascribed to a pollen hormone present in the pollinia. Now it can be shown to be produced by g. s. of the orchid pollen (40). G. s. also inhibits shedding of the leaf stalk after the leaf blade has been cut off (39, 71). To prevent the leaf stalk from being severed from the stem some g. s. is applied to the apical cut surface of the stalk.

## X

The rôle of the growth-hormone in plants is not limited to direct growth phenomena as described in preceding paragraphs. Processes depending on differential growth also are directed by it. Phototropism as well as geotropism are caused mainly by asymmetric distribution of auxin inside the bending organ. The same thesis can be defended with regard to epinastic movements (73). However, this is not the place to discuss these questions (see future article on Phototropism).

Still it might be worth while to point out that in elongating stem-like organs of the higher plants auxin is always connected with the growth process. This does not mean that all growth phenomena are auxin-phenomena; it only says that one definite part of each growth process is controlled by auxin. For this reason each student of plant growth ought to determine to what extent auxins are involved in the process under investigation. If they play a major part as in tropisms, auxin analysis will give a basis for explanation of the process; analysis of the production, transport and action of auxin furnishes a means of differentiating growth phenomenon into a number of factors, each one liable to yield a clue for a causal explanation.

"Bios" and "*B-Wuchsstoffe*," likewise, will not be treated here; they affect growth of yeasts, *i.e.*, increase in number of cells. Functionally they are more related to wound- and lepto-hormones. Their properties are completely different from auxin (among others, the latter is soluble in ether and "bios" is not).

## XI

As mentioned before, the best source of auxin is human (and mammalian) urine. This raises the questions whether this g. s. is synthesized in the animal body and whether it has any significance for its metabolism. Both questions have not been definitely an-

swered as yet. We owe, however, a number of interesting facts pertaining to the source of g. s. in the human body to the investigations of Kögl, Haagen Smit and Erxleben (33). It has been found that generally after meals the auxin *a* concentration in urine is increased. This is not correlated with intake of sugars or starch and proteins have but slight effect. However, after eating fat (butter, vegetable oil), excretion of auxin is enormously increased. This is not due to fat metabolism because hydrogenated oils do not have any effect; the hydrogenation has destroyed the auxin of the fat. In most cases auxin seems to be present in esterified form and is not active until it has been saponified.

Auxin content of urine except after meals is very constant and does not change materially upon fasting. Recently Kögl and co-workers (36) published evidence which makes it extremely likely that this "basal auxin" is hetero-auxin, mainly synthesized from tryptophane or allied substance by bacteria in the bowels.

A complete account of the source of all auxin in urine has not been given so that, as mentioned before, the possibility remains that it is synthesized in the human body (34). In connection with this possibility and in view of the considerable amounts of auxin present in all organs of mammals and man the question has been raised whether auxin has physiological significance in the animal organism. The fact in itself that auxin is present in carcinomata in about twice the concentration present in other parts of the body (31, 42) does not have any significance because its concentration in urine is many times higher still. In tissue cultures addition of auxin did not affect growth of fibroblasts. However, as serum, the culture medium, contains auxin this result is not conclusive.

As a general conclusion from the facts known we must say that there is no convincing evidence either in favor or against a synthesis or a physiological rôle of auxin in the animal body. The fact in itself that the presence of g. s. is easily demonstrated in extremely small amounts partially explains its discovery in animal tissues; this technical advantage, however, does not warrant any physiological importance of auxin for the animal.

Other reviews of this field are to be found in citations 2, 11, 22, 41, 51, 64, 74.

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# THE BOTANICAL REVIEW

VOL. I

JUNE, 1935

No. 6

## THE GYMNOSPERMS

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### INTRODUCTION

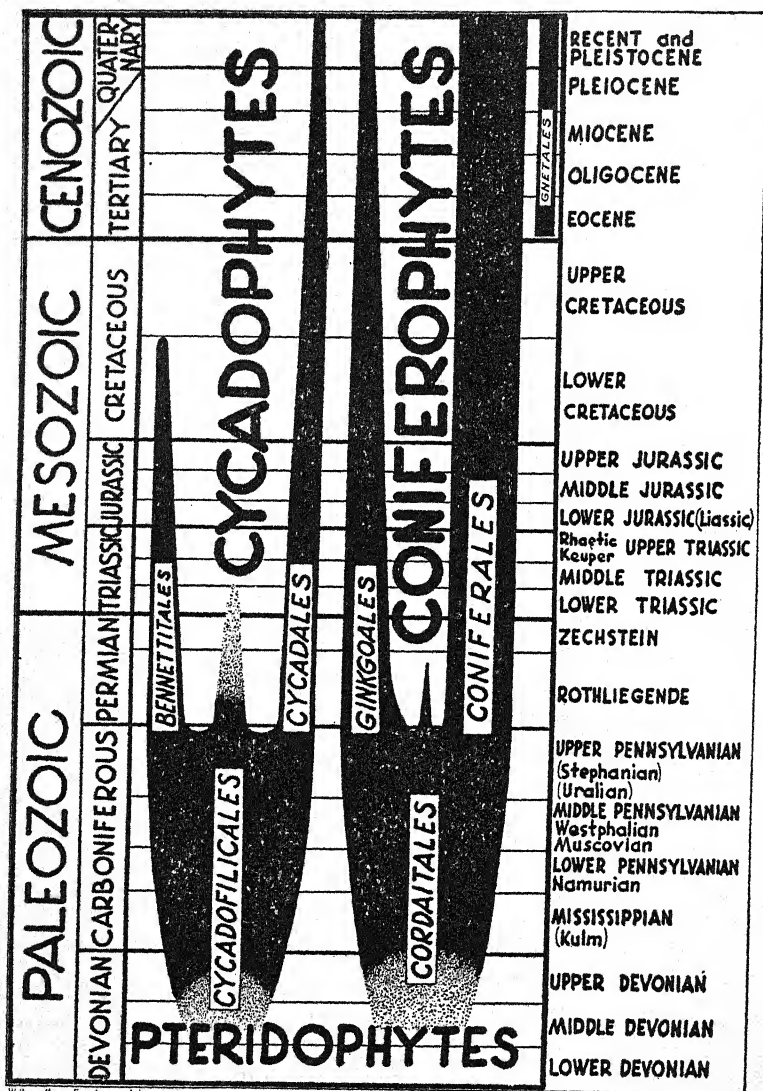
During the past thirty years, researches upon Gymnosperms have dealt with morphology, taxonomy, phylogeny and, to some extent, with physiology. The fact that they are the oldest of living tree forms, going back at least to the Devonian,<sup>1</sup> has made the group attractive to investigators. Most of their contemporaries of the lycopod line reached their culmination, both in size and number, during the Carboniferous and, at the height of their development, became extinct. The ferns also, certainly the heterosporous<sup>2</sup> ones, reached their culmination at the same time, and many became extinct. However, small survivors of the lycopod line, and both large and small survivors of the fern line are abundant today. Most researches, especially those of recent years, attempt to connect the living and extinct forms. For the origin of Gymnosperms we must look to these ancient plants.

Any relationship between Gymnosperms and Angiosperms is still indefinite. Angiosperms are being found farther and farther back, certainly in the Jurassic; but they are not so old as Gymnosperms. Robert Brown's definition of Gymnosperms as seed plants with ovules not enclosed in an ovary, and of Angiosperms as those with ovules enclosed in an ovary, while it made convenient pigeonholes in the herbarium, may have hardened ideas and made it difficult to interpret forms along the border line.

The number of genera and species of Gymnosperms is still increasing, partly by discovery of new forms, especially in the southern hemisphere, and partly by splitting forms already known.

Improvements in the microscope and in technique are resulting in more detailed cytological studies; and well preserved fossil

<sup>1</sup> See appendix for explanatory notes.



William Hurst Friedman del.

FIG. 1. Diagram indicating conjectural positions of the various gymnosperm groups in geological time. The horizons were compiled by Dr. A. C. Noé from various sources. The comparative amount of space does not equal the comparative amount of time on account of lack of space for the illustration.—From Chamberlain's *Gymnosperms*, The University of Chicago Press.

material, with constantly improving technique, is extending our knowledge of extinct forms. The fact that so much is being found out about fossils of the university zone of the northern hemisphere, gives hope that fossils of the southern hemisphere will fill equally great gaps in our knowledge.

The two great phyla, the Cycadophytes, comprising the Cycadofilicales, Bennettitales and Cycadales; and the Coniferophytes, comprising the Cordaitales, Ginkgoales, Coniferales and Gnetales, will be considered separately.

#### CYCADOPHYTES

All three orders of this phylum have received intensive study but many problems remain to be solved.

*Cycadofilicales*: In England the earlier work of Williamson has been extended, principally by Scott (87, 88), Seward (90, 91), Kidston and Oliver, with younger men like Gordon, H. H., Thomas, Walton and Harris now active in research. In Europe, the most prominent names are Stur, Renault, Ettingshausen, Potonié, Zeiller, C. E. Bertrand and Paul Bertrand; more recently, Gothan, Halle, Jongmans, Kübart, Kräusel (53) and Florin (35). In America, coal-balls<sup>3</sup> were not known until 1922, when Noé discovered them in various places and, with his students, began a series of investigations which indicate that American material is as abundant and as well preserved as that found in England and Europe. Noé's "Carboniferous Flora of Northern Illinois" (67) shows that many genera and perhaps even species are the same as on the other side of the Atlantic.

Earlier work, which consisted largely of description of scattered fragments of stems, leaves and roots, with a smaller representation of reproductive structures, is still continuing; and occasional pieces of leaves in connection with stems, and reproductive structures in connection with leaves, are enabling workers to assemble disconnected parts of life histories. Modern technique, especially the labor saving peel,<sup>4</sup> makes possible rapid exploration of material. Histological details are becoming known in such a wide range of material that they serve as well as impressions in making determinations and perhaps even better in guessing at relationships.

An investigation of cuticular structures, covering both Cycadophytes and Coniferophytes, has been undertaken by Florin (35).

Both living and extinct forms are treated. The first volume, with 588 pages, 58 plates and 111 text figures has already appeared. This work is bringing to light another important feature which must be taken into account in any theories of relationship.

While the structure of stems, leaves and roots of extinct forms is becoming fairly well known and some of the vegetative features of sporangia are coming to light, little is known of the gametophytes. Since some pollen grains have been found in mature pollen chambers<sup>5</sup> in contact with the female gametophyte, there could have been no pollen tubes. It might be taken for granted that motile sperms of Pteridophytes were retained by early Gymnosperms.

Megaspores of heterosporous Pteridophytes have been found in such prodigious quantities that they form an important part of some varieties of coal; but megaspores still within the sporangium are comparatively rare.

In early Gymnosperms the megaspore membrane is thinner than in heterosporous ferns. Some cellular structure of the female gametophyte has been found but no well defined archegonia. The most surprising thing is that, with the great number of fairly well preserved seeds which have been sectioned, not a single embryo has yet been found. Some of the seeds, like *Trigonocarpon*, must have reached their full size, for they are several centimeters in length, the contained gametophyte seems to have reached maturity and microspores are in contact with it. These later stages are the ones usually found and they probably represent the culmination of the order. The earliest stages in transition from heterosporous Filicales to lower Gymnosperms are still to be described. Early seeds must have been very small and resemblance between ferns and early Gymnosperms almost indistinguishable in reproductive characters as well as in leaves and histological features of the stem. Dr. Fredda D. Reed (73) found in the Paleozoic lycopod *Bothrodendron* a megaspore as far advanced toward the seed condition as is the living *Selaginella apus*. With a little thicker sporangium wall and permanent retention of the already long contained megaspore, these lycopods would have attained the seed condition.

When the evidence is all in, we feel sure it must show the sequence, homosporous Pteridophyte, heterosporous Pteridophyte, seed plant.



*Bennettitales*: Since Wieland's (103, 107) classic work on the Bennettitales, nothing of even approximate importance has appeared. Investigators seeking material in regions where he had collected found nothing which he thought worth taking; and no new collecting grounds of equal importance have been discovered.

However, work on the Bennettitales is not yet complete. There is certainly good material of both the Williamsonia and Cycadeoidea groups in southern Mexico; and such material is in regions where living cycads are still to be found. As a promising collecting ground let us suggest the southern part of the *Dioon spinulosum* region, about 100 miles south of Vera Cruz; and the region to the west, on the other side of the mountains.

Wieland's work was devoted, chiefly, to the Cycadeoidea section of the group, the work on the Williamsonia section being largely from impressions. Both the ovulate and microsporangiate structures of the Williamsonias need much more study. The relation of the ovulate strobilus to the stem, while presumably like that in *Dioon*, is still to be determined. Gametophytes in the whole order, as in all extinct material, are still in need of accurate description. Since other structures, equally delicate, have been preserved, it must not be imagined that this part of the life history can not be found.

*Cycadales*: The Cycadofilicales became extinct in the early Triassic, the Bennettitales in the middle of the Cretaceous; but the Cycadales, once world-wide in their distribution, still survive in a few tropical and subtropical regions. Since they are easily recognizable in the Permian and still exist, there must have been a continuous flora throughout the Mesozoic. Occasional fossil specimens have been found, but not in abundance; and the record is almost entirely from impressions. However, their leaves and stems were so like those of the Bennettitales that many of the structures assigned to the Bennettitales may really belong to the Cycadales. Cones of the Bennettitales are mostly axillary and well protected. Consequently, they are generally preserved. Although there are some axillary cones in Cycadales, most cones are terminal and even axillary cones have little protection at maturity. Cycadales cones usually become very soft at maturity and soon decay, so that preservation in the form of petrifications or even impressions is comparatively rare.



Studies on living forms have dealt with every phase of the life history. Chrysler (19) went to Cuba to study *Microcycas* in the field and to collect material for microscopic investigation. He found that the stem is monoxyletic,<sup>6</sup> with distinct growth rings and that it has cone-domes<sup>7</sup> like those of *Dioon*. The xylem of the stem is endarch,<sup>6</sup> consisting typically of pitted tracheids,<sup>8</sup> but with a wide zone of scalariform<sup>8</sup> tracheids bordering on the pith. Where recognizable, the protoxylem<sup>8</sup> has narrow spiral<sup>8</sup> tracheids, followed by reticulate and then scalariform elements. In the peduncle of the male cone there is some centripetal<sup>6</sup> xylem, making the bundles slightly mesarch.<sup>6</sup> From a comprehensive study of the anatomy of the stem, leaf and peduncle, Chrysler believes that *Microcycas* is a very advanced form and not primitive as one would conclude from study of the male gametophyte.

Dr. Sister Helen Angela Dorety (26) traced the vascular system of several cycads, especially in seedlings. Her work on the seedling of *Dioon spinulosum* deals with the girdling,<sup>9</sup> so characteristic of cycad stems.

Dr. La Dema M. Langdon (59), also studying *Dioon spinulosum*, found the relation between leaf gaps and leaf traces<sup>10</sup> and showed how the girdle is built up by contributions from many leaf gaps.

Dr. Sister Mary Alice Lamb (58) investigated the leaves and leaflets of all genera and many species. They are so characteristic that she constructed a key, based upon leaflets alone. Miss Sophia Papadopoulos (68) made a comparative study of the leaves of *Ceratozamia mexicana* and *Zamia monticola* and of a hybrid between them. The cross was particularly favorable, about 50 vigorous F<sub>1</sub> plants having developed from the seeds. In topography and in histological characters, especially the stomata, the hybrid resembled both parents.

Dr. E. Grace Smith (96) traced the vascular connections at the apex of the stem in *Zamia floridana*, particularly in cases with numerous cones. She found stems with as many as 10 staminate cones. Usually, when there are more than two or three cones, the stem branches, so that there are only a few cones on a branch. Any cone is terminal on its own branch and brings the life of that branch to an end; but a meristem, or two meristems, may appear at the base of the peduncle and give rise to cones. Meristems may

appear in rapid succession, each one giving rise to a cone. In some cases, multiple cones are due to adventitious buds in the cortex.

One of Lawson's (60) last researches was a life history study of the Australian *Bowenia serrulata*. The prothallial<sup>11</sup> cell is not so persistent as in the other cycads and it does not press into the stalk cell.<sup>11</sup> At the first mitosis in the fertilized egg, there are two distinct spindles, one from the egg nucleus and the other from the sperm. There are only 6 free nuclear divisions in the fertilized egg before walls appear, the lowest number ever reported in a cycad and a number which is sometimes reached in the conifers.

Sedgwick (89) found that in *Encephalartos* there is a very strong prothallial cell which, with the stalk cell, presses into the body cell.<sup>11</sup> At the ventral canal<sup>5</sup> mitosis, no wall is formed between the ventral nucleus and that of the egg. The ventral canal nucleus often enlarges and fertilization of the egg by this nucleus is practically certain. Some material of *Encephalartos Altensteinii* from Fairmount Park, Philadelphia, where there was no possibility of the usual fertilization, gave strong evidence of fertilization by the ventral canal nucleus.

Dr. Dorothy G. Downie (30) visited the Pinar del Rio region of western Cuba and pollinated *Microcycas*. Plants are so widely scattered that well pollinated cones are rare. She found that the numerous sperms in this species come from repeated divisions of the so-called stalk cell, so that the stalk cell is really spermatogenous. This interpretation, which is certainly correct for *Microcycas*, suggests that the stalk cell, so generally present in Gymnosperms, may be, phylogenetically, spermatogenous, like the body cell.

Dr. Lillian Reynolds (74), studying the female gametophyte of *Microcycas*, found several groups of archegonia, on the top, on the sides and even on the bottom of the gametophyte; but only the group at the top has an archegonial jacket<sup>5</sup> and even this one is not highly developed. This is also the only group which has an archegonial chamber<sup>5</sup> and the chamber is shallow, not nearly so deep or clear-cut as in the other cycads. There are 64 or more archegonia. The number is difficult to determine on account of uncertainty in distinguishing between incipient archegonia and larger vegetative cells of the gametophyte.

Kuwada (56) investigated staining reactions of the sperm and egg of *Cycas revoluta* and, with Maeda (57), structure of the protoplasm around the blepharoplast.<sup>11</sup> In the same species Nakamura (66) counted chromosomes. While he found 12 in the female gametophyte and 24 in the nucellus,<sup>5</sup> he reports 11 in 132 cases out of 140 at metaphase of the first division in the pollen mother cell. In 8 cases the number was 12, as reported earlier by Ishikawa. Although the chromosomes are of various sizes, two gemini are noticeably larger than the rest.

In taxonomy there have been notable contributions. Pilger (71) wrote the entire Gymnosperm section of the second edition of Engler and Prantl's "Die Natürlichen Pflanzenfamilien." Schuster (85), in much greater detail, monographed the Cycadaceae for Engler's "Pflanzenreich." Schuster's strictly taxonomic section (in Latin) is preceded by sections on geographic distribution and life histories. The work brings together an immense amount of information which will be useful for reference; but it has the air of the herbarium rather than of the field. Morphologists have little interest in sub-species, varieties and forms, some of which may never occur again except that they show how much a species may vary. In this monograph, species are put together, which a field study would have separated; and species are separated which a field study would have put together. However, the work is a scholarly assembling of the morphology, geographic distribution and classification of the cycads.

Scott's (87) "Studies in Fossil Botany" has become a permanent part of the literature of Gymnosperms; and his "Extinct Plants and Problems of Evolution" (88) presents the subject in a thoroughly scientific manner and yet within the reach of those not specially trained in paleobotany.

Seward's (90) "Fossil Plants" has also become a permanent part of the literature and his "Plant Life Through the Ages" (91) traces the evolution of plants from their earliest records through the geological periods. It is intended for about the same readers as Scott's "Extinct Plants" but is more extensive and more detailed.

My own book (18), just published, has chapters dealing with field conditions, life histories and phylogeny of cycads.

## CONIFEROPHYTES

The living members of the Coniferophyte phylum have received more attention than the Cycadophytes because they are dominant in the university zone of the northern hemisphere and material is easily secured. Besides, south temperate material is becoming available. The books of Scott (87, 88) and Seward (90, 91) furnish an excellent introduction to the extinct members of the line.

*Cordaitales*: All investigators agree that this order can be recognized with certainty in the Lower Carboniferous; but how far below that horizon or how far above, depends upon interpretations of araucoid<sup>12</sup> stems in the Devonian and leaves in the Triassic and Rhaetic. Kräusel (53) thinks that these lower horizon stems and higher horizon leaves cannot be assigned to the Cordaitales with any certainty; while Wieland (103-107) believes that the group goes back to the Silurian, developing into great forests in the Devonian, culminating in the Carboniferous, declining in the Permian and becoming extinct in the Triassic.

In the Devonian, practically nothing but stems is available. In nearly all cases, it seems safe to assign a piece to the Coniferophyte or Cycadophyte lines but the older the material the less marked the differences between the two lines become. Whether this means that both came from common stock or that similar conditions resulted in similar structures is still to be determined.

Florin's (35) work on cuticular structures, already referred to in connection with cycads, adds much new data which seems to be of value in determining relationships. The structure and development of stomata are illustrated and described in great detail in living Coniferales and compared with those of Cordaitales. References to his numerous papers on fossil forms are given in this book. The comparison shows definite resemblances between cuticular structures, especially stomata, of the Cordaitales and lower Coniferales, and supports the theory that the Coniferales have come from the Cordaitales or, at least, that both have come from a common stock.

Coal-balls collected by Noé in various places in Illinois are being examined and some of them contain fairly well preserved seeds of Cordaitales. There are also excellent impressions of reproductive structures (67). Dr. Fredda D. Reed has sectioned some of the seeds. They show a well preserved testa and nucellus, a strong

megaspore membrane and some cellular structure of the female gametophyte, but no archegonia or embryos. A full account will soon appear in the *Botanical Gazette*.

While stems, leaves, roots and reproductive structures have been found, leaves and stems in considerable abundance, and there has been some assembling, there is no such complete assembling of a life history as in *Lyginopteris*.

At present, it is safe to say that, so far as evolution of the vascular system is concerned, the Cordaitales have advanced further than the Cycadofilicales. Even the earliest reproductive structures of Cordaitales are cones, a stage in evolution not reached by any of the Cycadofilicales.

*Ginkgoales*: During the past 30 years not much has been added to our knowledge of fossil members of this order. The range has been extended and a few sections of vegetative structures have been described; but internal structure, microsporangia, megasporangia, seeds and, especially, gametophytes, still remain to be investigated.

In the second edition of Engler and Prantl's "Die Natürlichen-pflanzenfamilien," Pilger (71) described *Ginkgo* and *Baiera*; and Kräusel (53) lists other extinct members. The principal investigations upon the living *Ginkgo biloba* have been by Dr. Stephanie Herzfeld (46, 47), Mann (63) and Shiamura (92).

Herzfeld, in two papers, studied field conditions, fertilization and embryogeny. It is well known that male and female strobili have been found on the same tree. Many botanists have seen the monoecious tree at Kew; but the monoecism is due to a successful graft. She mentions other grafts but thinks that the monoecious condition sometimes occurs naturally. We have never seen it and we believe that any claim must be thoroughly supported.

There are two trees in the Royal Garden at Rome which bear good seeds, although there are no male trees in the city. She also cites a tree at Germantown which bears good seeds, although the nearest male trees are six miles away. Since fertilization by the ventral canal cell is known to occur in *Picea*, *Pinus*, *Abies* and even in *Ginkgo*, it is not necessary to regard these cases as parthenogenetic.

In material collected at Vienna, she found 12 bivalent chromosomes of various sizes at the metaphase of the heterotypic mitosis

in the microspore mother cell. One of the chromosomes is definitely larger than the others and many times the size of the smaller ones. The same number was counted at the ventral canal mitosis.

The most detailed part of her work concerned fertilization and early embryogeny. At the first mitosis in the fertilized egg there are two distinct spindles, one developed from the nucleus of the sperm, the other from that of the egg. In each of the figures the  $x$  number of chromosomes was counted. Two distinct spindles were noted by Lawson (62) at the first mitosis in the fertilized egg of *Bowenia*; and, in some conifers, considerable independence of male and female chromatin has been described; but in *Ginkgo* she found the independence persisting in one nuclear generation after another, up to the seventh simultaneous free nuclear division, nearly through the entire free nuclear period, for there are only 256 free nuclei. Later, there is only one spindle and the mitoses do not seem to present anything unusual.

Six years later, Shiamura (92) studying, principally, material from the famous tree from which Hirase made the collections proving that *Ginkgo* has motile sperms, confirmed Herzfeld's count of 24 as the  $2x$  number of chromosomes; but neither his description nor his numerous photomicrographs shows the paired spindles. The spindles appear broad and truncate but not double.

Mann (63) investigated reduction divisions in microspore mother cells, paying particular attention to the behavior of plastids. During prophase of heterotypic division, the plastids enlarge and some of them divide. As the telophase is reached, the plastids become arranged in two rings at the centripetal poles of the two nuclei, with approximately half of the nuclei accompanying each nucleus. At the close of the second division, the plastids are in four rings, with similar arrangement. Near the close of the second division, the outer wall pushes in between the nuclei, a process described as furrowing. When it is completed, the formation of spore coats begins. Mann believes that plastids have something to do with formation of the cell wall.

Sprecher's (98) book, dating back to 1907, is still the most complete account of the life history of *Ginkgo*.

During the past 30 years nothing has been found to weaken the theory that the Ginkgoales have come from the Cordaitales.



*Coniferales*: During the past 10 or 15 years, research in the *Coniferales* has been more persistent and widespread than in any other order of *Gymnosperms*. More than one-third of the papers and books listed in our bibliography deal principally or entirely with this order. There have been important contributions to all phases of life histories, comparatively inaccessible genera have been described and gaps have been filled in where accounts were fragmentary. Especially prominent among investigators who have worked on life histories are Buchholz (3-11), Hutchinson (50), Lawson (60, 61), Saxton (78-81) and Dupler (31), with text book accounts by Schnarf (82), Schürhoff (84) and myself (18). These books cover practically the entire life history and have very full bibliographies.

Covering the entire field of paleobotany are the indispensable books of Scott (87, 88) and Seward (90, 91). From the standpoint of taxonomy, Pilger's (71) revision of *Coniferales* for Engler and Prantl's "Die Naturlichenpflanzenfamilien" is the most important. In addition to the taxonomy, he presents a review of morphology and an extensive treatment of geographic distribution. The first volume of Florin's (35) comprehensive investigation of cuticular structures of both living and fossil forms is also of first importance.

The most recent book on paleobotany is Dr. Elise Hofmann's "Paläohistologie der Pflanze" (48). It covers the whole ground, from algae to *Angiosperms*, with about one-third of its 308 pages devoted to *Gymnosperms* and half of the *Gymnosperm* section devoted to conifers. It is dominantly histological; many illustrations are new. The main thesis is that plants can be identified by their histological structure. Some lower conifers, related to *Cordaites*, have histological structures similar to those of the *Cycadofilicales*. She believes that histological structures, especially pitting, indicate that the *Araucariaceae* are the oldest of the conifers, the *Abietaceae* coming later. In this connection it must be remembered that investigators may agree upon an evolutionary series of structures and differ as to what is the beginning and what the end of the series.

Bailey's (1) recent book deals with cultivated forms. Although the title is "The Cultivated Conifers of North America," the work will be almost equally valuable throughout the entire north tem-



perate zone. Cultivated conifers are often hard to diagnose, but with this splendidly illustrated book, determinations can be made with confidence. The author's long experience, especially with cultivated plants, has kept him from laying undue stress upon fluctuating variations which may never occur again; and his wide acquaintance with conifers in the field has enabled him to bring out differences between cultivated plants and those growing wild. Morphologists have paid considerable attention to abnormalities like bisporangiate cones and have noted them more frequently in cultivated specimens. The question might be raised whether abnormalities are really more frequent in cultivated specimens, or whether they are not merely more likely to be observed.

Pilger (71) recognizes seven families, in the following order: Taxaceae, Podocarpaceae, Araucariaceae, Cephalotaxaceae, Pinaceae, Taxodiaceae and Cupressaceae. In our own account (18) we have put the taxads and podocarps at the end of the series instead of at the beginning.

If the geological record were complete, the sequence of families would be settled by the sure testimony of history; perhaps not definitely settled, because monocots were abundant in the Carboniferous, until the "parallel" veined leaves turned out to be leaves of Cordaitales. Records in the rocks are still too fragmentary, especially in reproductive structures, to settle the arrangement.

If structure of wood be made the basis of classification, the Araucariaceae bear such close resemblance to the Cordaitales that, in many cases, determinations may not be convincing. But the Abietaceae also show some very primitive characters, perhaps antedating the Araucariaceae. If the amount of prothallial tissue in the male gametophyte be made the basis of classification, the Araucariaceae would easily stand first with Abietaceae further along and the Cupressaceae near the end. But the Cupressaceae have very primitive sperms and, some of them, numerous sperms, not far removed from the ancient swimming condition. In this feature, the Abietaceae would have a median position with the Araucariaceae still more advanced. The male strobili of taxads are of an ancient type; but the inequality of the sperms is an advanced condition and the ovulate structures, in our opinion, are near the end of an evolutionary series. If embryogeny be made the basis of classification, there would be still another sequence.

The rapidly accumulating knowledge of life histories of living forms and the gradual increase in knowledge of extinct forms is likely to determine whether the taxads should come at the beginning or the end of the series and may determine which is the more ancient, the Abietaceae or the Araucariaceae.

Of both scientific and human interest, is the work of Douglass (27-29) upon annual rings. He studied, principally, the rings of *Sequoia* and other conifers. The chief result was that the ring of a dry year differs from that of a wet year. A series of dry years is marked by small rings while, in wet years, the rings would be larger. By cutting a living tree and noting the sequence of rings of various sizes, a piece of timber from approximately the same locality, cut hundreds of years ago, can be dated with considerable confidence. It seems probable that the Puebla ruins of our southwest may be dated by timbers used in their construction. Of course, a piece of timber might be cut sometime before it was used but the dating would be approximately correct. Ever since Douglass made his first report in 1909, there have been accounts of rings, at first concerned more with determining the age of the large Sequoias, some of which are 4000 years old; but later, anthropologists have become much interested in this phase of botany because it seems to offer the most reliable data for determining the age of ancient buildings of cliff dwellers.

In histological structure, there is still some work on ray tracheids,<sup>8</sup> but they seem to be confined to the Abietaceae. Some interpret them as modified cells of the ray while others think they are modified tracheids. There have not been such detailed investigations of the anatomy of southern hemisphere forms, especially some of the more inaccessible ones, as has been given to familiar north hemisphere species.

The bars and rims of Sanio<sup>8</sup> have received some attention and have been found in all families of conifers except the Araucariaceae and, even here, they occur in the cone axis of *Araucaria*. Since this is a conservative region, it is assumed that the bars were formerly present in the wood. So, since they do not occur in the Cordaitales, the Cordaitales could not have been the ancestors of the Araucariaceae. While a cytological study of the origin of the vascular system of the ancestors of the conifers cannot be hoped for, such a study of living forms might help in determining their relationship to each other.

Florin's (35) extremely comprehensive study of cuticular structures, the first volume of which, dealing with Cordaitales and Coniferales, has already appeared, will at least add another character for determining relationships. The stomata and their development are treated in great detail. Ingenious technique, especially in dealing with carbonized material, has enabled him to make efficient studies of extinct forms.

Saxton (78-81), long a student of Gymnosperms, has added some later stages of the ovule of *Saxagothea* and has given the first life history account of *Austrotaxus*, an interesting new genus from the northern part of New Caledonia. The ovule is terminal and strongly resembles that of *Taxus*. Sporogenous cells are numerous and there is a tendency for more than one female gametophyte to develop, as in *Taxus*. The neck of the archegonium has as many as 16 cells in one tier. There is a ventral canal nucleus, but not a ventral canal cell. The male strobilus is very striking and, in longitudinal section, bears close resemblance to the reproductive branch of *Psilotum*, with a trilocular sporangium in the axil of each sporophyll. However, the sporangia of *Austrotaxus* are terminal, in a fused group of three, not pendulous from a peltate expansion; while in *Psilotum* the trilocular condition is believed to be due to sterilization of sporogenous tissue. Saxton objects to the term "peltate sporophyll" for either *Taxus* or *Austrotaxus* because a leaf cannot be borne in the axil of another leaf. The *Austrotaxus* cone is more primitive than the *Taxus* type and, consequently, could not be derived from it. The ventral canal nucleus divides, so that there may be as many as 7 nuclei at the top of the egg. In early embryogeny there are 8 nuclei before any walls appear and even then some of the nuclei do not divide until the next division. As differentiation proceeds, there are four tiers, an upper tier of free nuclei, a rosette of 9 cells, a suspensor of 9 cells, while the lowest tier consists of only one cell. There is no cap to be cast off later as in *Cephalotaxus*.

Saxton and Doyle (81) described the embryo and gametophyte of *Arthrotaxus selaginoides*.

Hagerup (39) secured both male and female strobili of the Sumatran *Dacrydium elatum*. In both strobili the sporangia are on the upper, adaxial face of the sporophyll, so that there is a striking resemblance to the position of sporangia in the lycopods.

He regards the strobili as inflorescences of the ament type and would apply the same interpretation to the rest of the conifers, except the Taxaceae and some of the junipers. He regards the integument of the conifers as a megasporophyll. With this interpretation, the megasporophyll (integument) of the conifers is homologous with the sporophyll of the lycopods, but not at all with the sporophyll of the Cycadophytes. Consequently, he thinks the cycads and conifers are too widely separated to be placed together in the Gymnosperms. *Juniperus*, in his opinion, stands nearest the Angiosperms.

The series of papers on *Taxus* by Dupler (31) gave a good account of the life history of this critical genus, especially of its gametophytes. The taxads are often referred to as "conifers without cones." A thorough investigation of floral development where fruiting is particularly abundant might show a female strobilus resembling a cone. If so, the present structures are very far removed from such an ancestral condition. However, it must be admitted that the male strobilus is very primitive.

A recent paper by Beal (2) deals with behavior of chromosomes at the first mitosis in the fertilized egg of *Pinus banksiana*. He finds two groups of chromosomes, as others have found them, and finds that the two spindles quickly merge into a multipolar diarch spindle with the chromosomes of the two gametes indistinguishable from each other. Each chromosome splits longitudinally and 24 chromosomes pass to each pole. He finds none of the pairing and transverse splitting described for *Abies balsamea* by Hutchinson (50). The two accounts do not agree; both may be correct but more investigation is desirable. At the present time, Haupt has an investigation of fertilization and early embryogeny in several California conifers well under way.

The most extensive work in recent years on morphology of conifers is a study by Buchholz (3-11) of the embryogeny of nearly all genera. With new and ingenious technique, he removes the embryos entire and uninjured. With such preparations, many examined in both living and fixed conditions and all of them after fixing and staining, it was easier to observe and safer to interpret, especially in the numerous cases of polyembryony. Where only one embryo comes from each of two or more fertilized eggs, the condition is called simple polyembryony. Where more than one

embryo comes from a splitting of the product of a single fertilization, the term cleavage polyembryony is used. In cleavage polyembryony (9) two types may be distinguished, determinate and indeterminate. In the indeterminate type, no embryo seems to have any advantage over the others during early embryonic competition. In the determinate type, one embryo, usually the terminal one, is more favorably situated and is usually the successful one. *Pinus*, *Biota*, *Chamaecyparis* and *Cryptomeria* have indeterminate cleavage polyembryony. *Pseudolarix* and *Dacrydium* furnish examples of the determinate type. In making such an extensive study, with as much still to be published as has already appeared, Buchholz found it desirable to make these distinctions.

As long ago as 1902, Coker noted that the terminal cell and, later, a group of cells of the early embryo, are binucleate. Buchholz found the same condition in *Dacrydium* and, in a forthcoming paper, will describe the same condition in a dozen more species of the Podocarpaceae, so that all the members of this family, except *Saxagothea* and *Microcachrys*, probably have binucleate embryonic cells.

At a somewhat later stage than that in which binucleate cells appear, an apical cell<sup>14</sup> was noted long ago. Buchholz has found an apical cell in *Pinus* where it cuts off segments as in Pteridophytes. He also found apical cells in *Sciadopitys*, *Thuja*, *Juniperus* and others. Forms without cleavage polyembryony, like *Picea*, *Larix* and *Abies*, and those with caps, like *Araucaria* and *Cephalotaxus*, have no apical cells. After a few segments have been cut off, further development is by a group of initials, a kind of transition which is well known in Pteridophytes.

Buchholz believes that polycotyledony<sup>15</sup> is primitive and that a lower number of cotyledons is due to fusion; while Hill and de Fraine believe that dicotyledony is primitive and that larger numbers are due to splitting.

The origin and early development of the vascular system has received little attention, because morphologists stop with the mature embryo in the seed; and anatomists do not begin until the vascular system is rather well established.

The number of families of conifers and their sequence will probably be changed many times before there is any general agreement. It seems safe to say that the order has come from the Cordaitales; what progeny it may have is still problematical.

*Gnetales*: The three genera of the *Gnetales* are so unlike that Markgraf (64) in the second edition of Engler and Prantl's "Die Natürlichen Pflanzenfamilien," makes three families, *Ephedraceae*, *Welwitschiaceae* and *Gnetaceae*.

The most extensive investigation of recent years and the one containing the most original work is the posthumous book, "*Gnetales*," by Pearson (69). The book was well under way when he died in 1916. Professor A. C. Seward, with the cooperation of Mrs. Thoday, completed the work, with the aid of Prof. Pearson's notes, and the book was printed in 1929. It covers geographic distribution, taxonomy, life histories and relationships.

Herzfeld (46, 47) made a detailed investigation of fertilization and nearly related stages in *Ephedra*. She described fertilization of the ventral canal cell by one of the sperm nuclei and fertilization of the egg by the other. The product of the fertilization was two groups of free nuclei. She did not hesitate to regard this as double fertilization in the *Angiosperm* sense.

A thorough investigation of *Ephedra* by Dr. S. Flowers with an abundance of well fixed material is now well advanced. This work will supplement the classical study by Land.

The origin of the *Gnetales* is still obscure. No reliable fossils have been found below the Tertiary and it is just as indefinite whether they have any progeny. *Ephedra* has unmistakable *Gymnosperm* characters and the other two genera resemble *Ephedra* in many ways, so that the three appear to belong together.

In the evolution of the vascular system, all three are very advanced, with vessels in the secondary wood, and *Gnetum* may have companion cells<sup>18</sup> in the phloem. The floral diagram of the male flower of *Welwitschia* bears striking resemblance to that of a tetracyclic *Angiosperm* flower and has led some to claim that the *Gnetales* are the ancestors of the *Angiosperms*. The evolution of the gametophyte, especially of the female gametophyte, is very advanced, with *Gnetum* almost in the *Angiosperm* condition. In general habit, *Gnetum*, with its netted veined leaves, would be mistaken for a dicot by any one except a botanist. But the fact that there are resemblances to *Angiosperms* does not prove that the *Gnetales* are becoming *Angiosperms* or that they have given rise to any *Angiosperms*. It can only be claimed that, developing along fundamental lines of evolution, they have advanced almost



as far as the Angiosperms. Even the most primitive forms are so advanced that they must have had a much more remote ancestry than has yet been discovered.

*Phylogeny:* Morphology, physiology and genetic relationship should precede taxonomy if taxonomy is to be anything more than a series of artificial keys for finding out the names of plants.

Similar structures are still the basis of all schemes of classification, but in phylogeny similar conditions may be responsible for many similar structures. Vegetative structures most intimately concerned in the life of the plant are likely to be the first to respond to changing conditions. Leaves are particularly responsive. Stems might be mentioned next. Gametophytes and early embryogeny may be the most stable features because they are less exposed to the struggle for existence. The embryo of *Lilium*, in all parts of the world, in warm or cold regions, in wet or dry conditions, is developed from four megaspores not separated by walls; while the stems, leaves and flowers show great diversity. The plant bodies of some euphorbias and some cacti are very similar, although there is no genetic relationship.

It is devoutly to be wished that more and better material of Paleozoic and Mesozoic forms be studied, especially gametophytes and embryogeny. Some vegetative material is so well preserved that one can reasonably hope that equally well preserved material of reproductive structures may yet be found. When records have become much more complete, such problems as comparative antiquity and origin of Araucariaceae and Abietaceae may be settled definitely; and we shall know whether to begin treatment of conifers with taxads or pines. Just now, from study of the literature, especially that of the past twenty years, there seem to be two strong lines, the Cycadophytes and Coniferophytes, both already highly developed in the Permian and differing so much from each other that they may not have had a common origin. From the Cycadofilicales, the Bennettitales and Cycadales have arisen independently. The Cycadales did not come from the Bennettitales. The Cordaitales, in our opinion, gave rise to the Ginkgoales and Coniferales. The Gnetales must have a more extended geological history than has yet been discovered; but at present, they seem to be a group with neither any yet discovered ancestry or progeny.



## APPENDIX

By the Editors

Readers especially interested in the Morphology of Gymnosperms are urged to consult Prof. Chamberlain's latest revision of his classic study upon the subject (citation No. 18). For those to whom this volume may not yet be available the following notes are here appended. They are presented only to furnish a general background for the accompanying article which discusses the entire group and necessarily involves terminology partly peculiar to the subject.

1. See fig. 1. for succession of geological eras.

2. *Heterospory*, the possession of two different kinds of spores, micro- and megaspores, is characteristic today of all seed plants, of the Water Ferns and of a few lycopods or club-mosses. It represents an evolutionary advance over homospority.

3. *Coal-balls* are calcareous nodules containing petrified patches of vegetable debris from the marshes and swamps of the closing stages of the Carboniferous period (Seward).

4. The *peel method* involves etching of a flat surface of material with acid and then flooding the surface with celloidin. The latter is subsequently peeled off, carrying remarkable detail of the embedded fossil.

5. The *megaspore* is the first cell of the female gametophyte. It is a cell of the nucellus of the ovule and is immediately surrounded by vegetative cells of the *nucellus*. It enlarges at the expense of these surrounding tissues and by successive nuclear divisions acquires many free nuclei, hundreds in some cases, which surround a large central vacuole. Wall formation then ensues about these nuclei and further centripetal development of tissue finally fills the entire original and now enlarged megaspore. The tissue, thus developed, constitutes the *endosperm* which, in this case, is the *female gametophyte*. At varying times during formation of this tissue certain superficial cells at the micropylar end, many in some cases, become *archegonial initials*. Except in *Welwitschia* and *Gnetum* each such initial enlarges and divides into an outer *primary neck cell* and an inner *central cell*. The former divides once in the cycads and *Ginkgo* producing two *neck cells*; in conifers the number is variable. The central cell then enlarges considerably and the surrounding layer of endosperm cells becomes differentiated as the *archegonial jacket* and performs some nutritive function for the contained *egg cell*. The nucleus of the enlarged central cell divides at a certain time to form the *ventral canal nucleus* and the *egg nucleus*. Adjacent cells of the endosperm may grow faster than the neck cells, pushing out the nucellus and forming a funnel-shaped *archegonial chamber* with the neck cells at its apex. This chamber is separated by the nucellus from the *pollen chamber* into which microspores enter through the *micropyle* or opening in the integuments which enclose all these structures. Microspores germinate in the pollen chamber, their tubes penetrate the nucellus and enter the archegonial chamber where they discharge swimming sperms among cycads and *Ginkgo* while in conifers they penetrate into the egg cell without formation of motile sperms.

6. Xylem is said to be *endarch*, *mesarch* or *exarch*, terms which refer to the radial direction of maturation of vascular cells with respect to the center of the axis, not to the center of the vascular bundle. If this progressive maturation, leading to differentiation of so-called *protoxylem* and subsequently to *metaxylem*, progresses away from the center of the axis toward the outside, growth is said to be *centrifugal* and the xylem is *endarch*. This condition prevails in stems of seed plants. If direction of maturation be toward the center of the axis, growth is *centripetal* and the xylem is *exarch*. This is true of all roots and of the axis of club-mosses; it is regarded as primitive. The intermediate condition, where maturation progresses both centripetally and centrifugally, is common in ferns and the xylem is then *mesarch*. If only one vascular cylinder differentiates from meristematic cells at the stem apex the stem is *monoxyle*.

7. *Cone-domes* appear in the pith and are groups of vascular bundles from the apex of which peduncles of cones arise. In longitudinal sections of the stem the bundles appear to form a convex diaphragm across the pith and in transverse sections as a circle of vascular bundles.

8. *Protoxylem* refers to the first cells to mature in the development of a vascular strand. The component tracheids have only *spiral thickening* which permits elongation; when thickening becomes *reticulate* or *scalariform*, in later maturing cells, further elongation is no longer possible. *Pitted tracheids* are the narrow, elongated, dead cells of xylem whose walls are thickened except for tiny, circular pitted areas. In most conifers special thickenings, known as *bars* or *rims of Sanio*, are located between pits on lateral walls of tracheids. In scalariform tracheids these thickened areas are narrowly elongated at right angles to the long axis of the cells. While tracheids are almost exclusively perpendicularly arranged in stems, in certain species some are horizontal, constituting part of the wood rays, and are then known as *ray tracheids*.

9. *Girdling* refers to the peculiar course of bundles which enter leaves of cycads. They join the vascular system of the stem, not near the base of the leaf but elsewhere, sometimes on the opposite side of the stem.

10. *Leaf gaps* are areas within the stems of ferns, gymnosperms and angiosperms where cortex and pith are continuous. They represent interruptions in the vascular cylinder where cells did not mature into vascular elements. They are located around and above the point of departure of *leaf traces*. The latter are prolongations of the vascular system of the stem into the leaves.

11. The uninucleate pollen-grain or *microspore* is the first cell in the *male gametophyte*. By successive divisions of the original nucleus a variable number of walled cells is formed within the microspore wall before the latter is shed from the microsporangium or pollen sac. In cycads the pollen is shed in a three-celled condition, one of the cells, the *prothallial cell*, being smaller than the others and representing the vestige of the prothallium in lower plants. This prothallial cell persists. In *Ginkgo* two such prothallial vegetative cells form, one of which disintegrates and the other persists as the single cell does in the cycads. In certain conifers

two such vegetative cells also appear in the microspore but both disintegrate. These stages represent evolutionary steps in decrease of vegetative tissue in the male gametophyte.

Another of the three cells in the cycad pollen, formed previous to shedding, is also small and known as the *generative cell*. After pollination and germination of the microspore within the *pollen chamber* of the ovule, this *generative cell* divides to form the *stalk* and *body cells*. The former of these is regarded as representing the stalk cell of an antheridium and the persistent prothallial cell in most cases presses into it. The *body cell* is the real spermatogenous cell. At a certain period a small structure known as the *blepharoplast* appears in it. The body cell later divides, along with the blepharoplast, into two sperm mother cells. Each of the latter produces a sperm with a spiral band of cilia developed from a blepharoplast. These motile sperms are the largest known among plants or animals.

Approximately the same sequence of events occurs in *Ginkgo* including the blepharoplasts and motile sperms. Body and stalk cells form from the generative cell also in conifers but blepharoplasts do not develop in the body cell and motile sperms do not form. The body cell divides directly, on the contrary, into two non-motile male cells (or only nuclei). A cell generation is thus significantly omitted, namely, the sperm mother cells of the cycads and *Ginkgo*.

12. Resembling *Araucaria*, a genus of conifers found now only in the southern hemisphere.

13. Except in the Gnetales, gymnosperm wood differs from that of angiosperms, among other features, in not having *vessels*. The latter are dead cells, usually wider than tracheids, which are joined together into tubes by disappearance of their end walls. *Companion cells* of angiosperms accompany conducting *sieve tubes* in the phloem and their nuclei are thought to serve the apparently enucleated sieve tubes.

14. Growing points of seed plants consist of many dividing cells. In ferns, on the other hand, all cells in these regions can be traced back to one *apical cell*.

15. Cycads and *Ginkgo* usually have only two cotyledons or seed-leaves per embryo. Conifers have a greater and variable number referred to as polycotyledony.

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## THE PRESENT STATUS OF THE PLANT ASSOCIATION

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### EDITORIAL PREFACE

The plant association has often been compared to the species. One point of agreement between them is that both have been subjects of strenuous discussion concerning their definition and scope. The species-question has been before us for more than a century, but has never been settled. Opinions still vary from the micro-species, consisting possibly of a single pure-line race, to the broad or Linnaean concept. The prevailing taxonomic usage of the term has been a matter of long development and is in general fairly well understood.

The association-question, on the other hand, was only recently thrust upon ecologists. They have attempted energetically to settle the various problems involved in it, generally by the advancement of individual opinions rather than by the slower method of gradual development through long experience.

The fact that definite societies or assemblages of plants exist in nature is an accepted fact. The proper interpretation of these societies, the terminology to designate them, and the general principles of their classification into larger vegetational groups are still matters of controversy.

What is a plant society? What is the underlying basis for its existence? Is it a concrete thing or an abstract concept? What is the fundamental unit of vegetation, corresponding to the species in taxonomy? Is it broad or narrow in its scope and by what name shall we call it? Can we group plant societies into larger complexes and can we logically recognize minor subdivisions of them?

Some of these questions are discussed by Dr. Pavillard in the present paper. The editors regret that it has been impossible to maintain the sparkling vivacity of the original French.—H. A. G.

### THE ASSOCIATION AS THE FUNDAMENTAL UNIT IN PLANT SOCIOLOGY

In the section of Phytogeography of the International Botanical Congress at Brussels in 1910, a serious attempt was made to reach

an international understanding on the concept of the plant association. The definition proposed by Flahault and Schröter deserves to be restated: *An association is a plant community of definite floristic composition, presenting a uniform physiognomy, and growing in uniform habitat conditions. The association is the fundamental unit of synecology.*

It is obvious that this statement consists of two parts. The first one has the character of a definition; it was not adopted by the Congress. The second has a systematic significance and assigns to the association a certain hierarchal rank and value; it was not particularly discussed at the meeting of May 20, 1910.

Now the importance of the first part of the statement clearly depends on the second, since in every experimental science the existence of a fundamental unit is the prime requisite for precision and exactness in the comparison and subordination of the subjects studied. If we admit that the association has this rôle of a fundamental unit, then we must also conclude that its most important characters are those which are most directly related to this rôle.

The oldest definition which reflects the importance of these characters was propounded by Du Rietz and his colleagues in 1920: An association is a plant society with definite constants<sup>1</sup> and definite physiognomy (22).

In this definition, the essential criterion and the only one which has been the subject of detailed study is the presence of a determinable number of constants. This number may be ascertained by the statistical application of the quadrat method,<sup>2</sup> and depends on the determination of the *minimum area*, which is the smallest extent of space which can be characteristic of each association.<sup>3</sup> In the minds of the Swedish authors, then, the recognition and delimitation of associations was a matter of rigorously precise procedure, which is alone capable of assuring the validity and comparability of groups worthy of being regarded as authentic associa-

<sup>1</sup> A constant is a species which is regularly present in every example of a plant association.—Editor.

<sup>2</sup> A quadrat is a small area of definite size, often one square meter, within which vegetation may be studied intensively. Results obtained from a sufficient number of quadrats yield important averages and may be used in statistical studies.—Editor.

<sup>3</sup> That is, it will contain the typical species and a fair representation of the others of secondary importance, and will in general be a fair sample of the whole association.—Editor.

tions. We shall refer later to the present fate of this method of work, so imperiously promulgated by Du Rietz in 1921 (14).

The practical result of the initiative of the Upsala group was to induce a certain number of Nordic ecologists and a few others to pulverize the vegetation of their respective countries into an innumerable multitude of minute rudimentary groups, under the name of associations, generally covering a very restricted area and corresponding accordingly to the lowest rank in the phytosociological hierarchy.

The publication of Du Rietz' voluminous dissertation opened an era of ardent polemics which are of no interest today. However, the arguments developed by Rübel in his philosophical essay of 1925 (62) merit brief consideration because of their ingenuity and their results. Attention would have been awakened differently in Sweden and in Switzerland by certain forms which are particularly frequent in the vegetation, said Rübel, and there would have resulted in the two countries a different concept of the association and a different technique. Study of the heaths covered by dwarf shrubs in Sweden would have led to micro-associations; study of the meadows of Switzerland would have favored macro-associations. There would thus have developed two parallel and possibly equally legitimate systems. Is it possible to unite them into a single system, or in other words to coördinate and subordinate the two kinds of associations, the small and the large?

Pursuing his efforts for conciliation and seeking to introduce new aspects into the argument, Rübel (63) returned to the question in 1927. Without ceasing to champion the classical partition of the association into sub-associations and facies characterized by differences in floristic composition, he considers the advisability of borrowing from the British and American ecologists another sort of subdivision based on physiognomic dominance.<sup>4</sup> Two new ranks, the *consociation* (12) and the *sociation*,<sup>5</sup> may thus be subordinated to the association, which still always maintains its dignity as the fundamental unit.

It is impossible not to recognize the effect of these suggestions by Rübel upon the new attitude adopted by Du Rietz in 1930, just

<sup>4</sup> See footnote on page 225.

<sup>5</sup> The term *society*, familiar to English-speaking ecologists since its presentation to the Brussels Congress by Tansley and Moss, is thus converted (fortunately?) by Rübel into *sociation*. Gams (25) gives the name *society*

before the Botanical Congress at Cambridge. His voluminous memoir in Abderhalden's Handbook (18) includes a reform project still more radical and extraordinary than his "laws" of 1920. Appropriating the terms consociation and sociation, Du Rietz incorporates them as inferior steps into an entirely new hierarchy for which he is alone responsible, although he seems erroneously to attribute the preliminary proposal of his innovations to Rübel.

The association still appears as the third stage<sup>6</sup> in his classification, but is now completely deprived of its systematic importance in favor of the sociation, which, according to Du Rietz, is the sole fundamental unit, alone analogous to the species, alone perfectly homogeneous and truly natural in all its parts. It is needless to say that such a concept could not be accepted by the majority of ecologists of central Europe.

At this time we shall not discuss or pass judgment on Du Rietz' system as a whole or on his terminology. The chief interest of his project, from our special point of view, lies in the definitions of the Swedish author and especially in that of the sociation, his fundamental unit, which is to be characterized by the presence of *constant dominants* in each stratum (layer) of the group.

In 1920 the association of Upsala, the exact equivalent of the sociation of 1930, was to be recognized, according to Du Rietz, chiefly by its *dominant constants*. Today we read *constant dominants*. Although this appears to be a simple inversion in the position and meaning of the words, it is not without its significance, and is in fact evidence of a complete and entirely unexpected reversal in the doctrine and ecological technique of Du Rietz.

Let us briefly recall what this technique was, according to his memoir of 1921: first, preliminary reconnaissance in the field of the probable associations according to the dominant constants; next, the necessary confirmation of this observation by determining the group of constants and the corresponding minimum area by the statistics of the quadrat method. That is the only method, said Du Rietz, through which definite and unimpeachable conclu-

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to aggregations of individuals of a single species or several species belonging to the same life-form. As the "fundamental unit of vegetation" the society must, according to Gams, be translated by *Bestand* as its equivalent term in German and Scandinavian.

<sup>6</sup> Ranking above the sociation and consociation.—Editor.

sions may be reached. How many times has Du Rietz claimed not only the superiority but the indispensability of his technique?

Of this Upsala method, so rigorous and precise, at least in appearance, nothing now remains. Not only does Du Rietz completely abandon his laws of constancy and his earlier definitions, but he now bases his diagnosis of the sociation on the dominants alone, asking for nothing else except a purely empirical floristic list as a source of supplementary information.<sup>7</sup>

The present purely empirical method of determining sociations justifies us in considering them as absolutely incapable of filling the rôle of fundamental units.

Du Rietz seems to have realized the serious inconvenience of the strange, almost paradoxical situation in which he placed himself. Less than two years after the Congress at Cambridge he discarded completely, although reserving for the future, most of his creations of 1930, especially the long series of *synusia*, which interest scarcely anyone. For the same reason he has also given up the attempt to secure official consecration<sup>8</sup> of his minor floristic units, the sociation and the consociation. For the Congress of 1935 he merely proposes a very limited program, the essential features of which concern the formation and the association. One can almost believe that he has gone back twenty-five years to the eve of the Congress at Brussels.

This is not the place to discuss the formation, which is not a part of our present subject.

In regard to the association, Du Rietz restricts himself to the proposal that a uniform use of the term should be agreed upon, applying it, if possible, to an ecological group of about the same rank as the associations of Braun-Blanquet and his followers. We are naturally quite disposed to rally round these propositions, but on one condition, which Du Rietz does not mention and which in our opinion should prevail above all others, and that is that

<sup>7</sup> What a curious spectacle is presented by some of these changes of attitude. After being converted to the technique of the Upsala school and having introduced it into Russia (36), and after earning the praises of Du Rietz (17), N. Katz (35) today places the statistical problems of the Scandinavian investigators and the so-called laws of constancy among the "ballast-problems . . . which do not repay the time and trouble devoted to them." According to Katz, the term association and its use as a fundamental unit should be applied only to groups of restricted extent, corresponding to the sociations of Du Rietz.

<sup>8</sup> I.e., adoption by a botanical congress.—Editor.



the association thus conceived should be capable of acceptance and recognition by all as the fundamental unit of plant sociology.

#### THE CONCEPT OF THE ASSOCIATION

What is meant by the term association? An important question, and an essential one for all who consider the association as the fundamental unit of plant sociology. Two conflicting theses, equally interesting, have been supported by A. G. Tansley and by H. A. Gleason.

Tansley (69) adopts from Clements the principal of the organic individuality of groups and develops extensive analogies between groups and living individuals; incidentally also between plant and human societies.<sup>9</sup>

In admitting "that vegetation forms natural units which have an individuality of their own," Tansley places himself among those investigators who have to "focus their attention on the vegetational units themselves." Now "the treatment of vegetation as consisting of natural units \* \* \* seems to involve their consideration as organic entities." "These units owe their existence to the interaction of individual plants of different species with their environment" and with one another (Cf. Yapp, 77). Undoubtedly "these natural entities \* \* \* are not organisms, in the full sense in which we apply the term to an individual animal or plant." But "it is clear that the aggregation of plant individuals which we call units of vegetation or plant communities have some of the characters which we associate with organisms."

An analysis of the general character of human societies leads Tansley to create for them the concept of "quasi-organisms." A comparative examination of vegetational groups permits him to conclude that "though less like true organisms than human communities, plant communities may still be regarded as quasi-organisms or organic entities, for on the one hand they are composed of organic units and, on the other, they are certainly entities, in the sense that they behave in many respects as wholes and therefore have to be studied as wholes."

This being admitted, "we must recognize \* \* \* that not all plant communities are of equivalent value. \* \* \* The organizing factors of vegetation \* \* give rise to various grades and kinds of aggre-

<sup>9</sup> Compare Harper (33).



gates. \* \* The general recognition of the association as the fundamental unit of vegetation implies that these organizing forces reach their highest expression in the association. The association is the most highly individualized vegetational entity."

Organization, interaction, interdependence then represent, according to Tansley, the essential elements in the concept of the plant association. We shall pass, for the moment, the supplementary criteria of maturity and stability, to which we may later have occasion to return.

Entirely different is the picture painted by H. A. Gleason (28). He admits as well founded "the numerous conclusions in synecology which depend directly upon observation or experiment. \* \* \* But our various theories on the fundamental nature, definition and classification of associations extend largely beyond the bounds of experiment and observation and represent merely abstract extrapolations of the ecologist's mind." Here we see, without doubt, the cause of the "apparently chronic inability" of the ecologists "to come to any general agreement on these matters." Nevertheless, "plant associations exist, we can walk over them, we can measure their extent, we can describe their structure in terms of their component species, we can correlate them with their environment, we can frequently discover their past history and make inferences about their future."

All methods which enable us to deepen our objective knowledge of associations are equally to be recommended, says Gleason. But not one of them gives us a solid foundation on which we may build a definition of an association: uniformity in the floristic composition of the vegetation is only an approximation, the correlation between environment and the association is marred by many irregularities, etc. Critical examination thus reveals to us the limited applicability, the doubtful exactness of all the characters generally employed in the definition and classification of plant communities.

In view of this total bankruptcy of facts, "are we not justified in coming to the general conclusion \* \* that an association is not an organism, scarcely even a vegetational unit, but merely a coincidence?"

Starting from the undeniable principle that "the phenomena of vegetation depend completely on the phenomena of the indi-

vidual,"<sup>10</sup> Gleason proposes to establish "the individualistic concept of the development of plant communities" as the only basis for the concept of the association. Any plant is capable of migrating in any direction by means of its seeds, provided that it meets with favorable environmental conditions. "Every species of plant is a law unto itself, the distribution of which depends upon its individual peculiarities of migration and environmental requirements. \* \* \* It grows in company with any other species of similar environmental requirements, irrespective of their normal associational affiliations. The behavior of the plant offers in itself no reason at all for the segregation of plant communities. In any general region there is a large flora and it has furnished migrating seeds for all parts of the region alike. Every environment has therefore had, in general, similar material of species for the sorting process. \* \* \* Since essentially the same environments are repeated in the same region, their selective action upon the plant immigrants leads to an essentially similar flora in each. The primary causes, migration and environmental selection, operate independently on each flora \* \* and have no relation to the process on any other area. \* \* \* The effect of these primary causes \* \* is to determine the plant life on every minimum area. The recurrence of a similar juxtaposition over tracts of measurable extent \* \* is due to a similarity in the contributing causes over the whole area involved."

"These conditions produce the well known phenomena of plant associations of recognizable extent and their repetition with great fidelity in many areas of the same region."

"Plant associations \* \* depend solely on the coincidence of environmental selection and migration over an area of recognizable extent and usually for a time of considerable duration. \* \* \* Similar conditions obtain for the development of vegetation in a new habitat." In general, chance plays a considerable part as well in the colonization of a new habitat as in the similarity of populations of "two areas of the earth's surface."

"Under the individualistic concept," and contrary to current opinions, in regard to associations, "the fundamental idea is neither extent, unit character, permanence, nor definiteness of structure.

<sup>10</sup> Starting from a similar principle, Ramenski (60, 61) concludes that associations do not exist and that their study is useless.

It is rather the visible expression, through the juxtaposition of individuals, of the same or different species and either with or without mutual influence, of the result of causes in continuous operation."

So Tansley says *organizing forces, competition, maturity, quasi-organisms*, while Gleason says *chance, migration, environmental selection, juxtaposition*.

Is it not possible that this complete antagonism suggests that both Tansley and Gleason have "forced their conclusions a bit too far?" Both of them probably have good reasons for the evidence which they use, but the conclusions which they draw are not so certain.

The question presents itself from a different angle to us of central Europe and we believe that it may be possible to bridge the chasm which separates the two concepts.

In its general form, the concept of the association implies the coexistence of a certain number of distinct species, living in common, occupying together a piece of habitable environment of large or small extent. Such a cohabitation, capable of almost identical repetition even at distant points of a considerable region, can not be the product of chance alone.

The most meticulous analysis, however, has not been able to demonstrate any evidence of altruism in plant societies. When one speaks of interdependence of organisms within such groups there is no thought of harmonious functioning or of a coöperation of diverse tendencies toward a common end for the collective welfare, as in civilized human societies. Each one for himself; that is the natural law in plant sociology. Each species finds itself there because of natural causes (migration and environmental selection) which permit it to be there, and each one maintains itself there because none of its neighbors is able to dislodge or suppress it. Each species, in general, is dependent on the other species only to the extent that it takes advantage of or profits by the environmental conditions which are determined by the presence of the others.

The term plant association, then, connotes a juxtaposition of specific elements which are strangers to each other and whose coexistence depends on their specific requirements, which may be

satisfied either by the conditions of the environment itself or by those conditions as modified by the presence of the other plants.<sup>11</sup>

Then if one association is succeeded by a second, it is not because one organism (Clements) or even one quasi-organism (Tansley) replaces the other. It is because certain species which have now become more privileged for one reason or another expel or destroy the original occupants; the displacement of the leaders reacts more or less fatally on their satellites (27). Reciprocally, it is certain that a definite, fully stabilized association, more or less in equilibrium with its environment, can never be established at once and *en bloc*, with all its floristic cortege. Like every natural phenomenon which is subject to the same law of evolution, the present state of a plant association, at any moment whatever, is only one stage, more or less advanced, in an ontogeny in which a whole series of individuals can participate.

Tansley scarcely speaks about anything except the reaction of the species on the environment. In our opinion, this is no more sufficient than is the simple elimination exercised by "environmental selection" according to Gleason. As a matter of fact, every species, no matter how modest or transient it may be, is not entirely without some power, we think, of reacting by its presence on the group of plants to which it belongs. This reaction on the whole assemblage must naturally be very variable in nature and intensity, but through it the society can find, in the course of its floristic development, a more or less efficacious, direct or indirect cause of consolidation and stabilization, just as it also finds a more or less menacing danger of disturbance and destruction. In short, every species is, we believe, a biological factor in the society of which it is a part.

In order to establish these ideas, I undertook in 1919 to draw attention to some of the factors in this sociological activity, distinguishing them as constructive, conservative, and destructive. These have since become familiar to a considerable number of European phytosociologists under the name of the dynamogenetic behavior of the species.

<sup>11</sup> Notwithstanding a statement which sometimes slips from the pen of even the most learned, a plant association is not a juxtaposition or cohabitation of organisms with the same demands upon the environment (i.e., the same ecology).

Dynamogenetic behavior is the direct or indirect reaction of the species simultaneously on the composition, the prosperity, and the internal organization of the plant society. In this domain it is probably the most important cog in the social machine. Research into the behavior of each of the specific elements and into their reciprocal reactions injects the dynamic principle into the very heart of the association-concept and thus demonstrates for this fundamental sociological unit the enlivening penetration of the Anglo-American method into the old static discipline of central Europe. We also remark in passing that the determination of the dynamogenetic behavior of species and societies results in placing autecology at the service of synecology.<sup>12</sup>

In conclusion, migration, environmental selection, and dynamogenetic behavior are the three bases which permit us not only to understand the genesis of associations, but also to comprehend and appreciate the differences which distinguish them from accidental and heterogeneous populations and from chance, unstable, and ephemeral juxtapositions. Thus the species depends on the population and the population depends on the species. Stability and equilibrium, realities which are the very essence of a plant society, imply a certain degree of interdependence and natural cohesion; these represent a bond of elementary solidarity which may legitimately be considered, we believe, as a particular type of organization.

Finally, from a philosophical viewpoint, the definite groups of which the association is the essential type appear to us to be stabilized combinations, endowed with a certain degree of permanence within their environment, but in their origin, their continued existence, and their ultimate natural disappearance subject to that universal principle of competition which prevails in all organic evolution, the struggle for existence.

#### ABSTRACT OR CONCRETE?

It appears certain that we can use for the association a language analogous to that used for the species, considering the latter as the fundamental unit in taxonomy. Such, in our opinion, is the philosophical basis for the comparison, made so often and from

<sup>12</sup> The so-called new trend of phytosociology, discerned (?) by Gams (26), implicitly followed (?) by Lippmaa (38), and discovered (?) by Katz (35), does not represent anything new.

such diverse viewpoints, between the association and the species. For example, various analogies have been invoked by Nichols in his interesting paper (43) devoted to a criticism of the individualistic concept of Gleason. And as Du Rietz remarked (15), the analogy between species and associations has often been used and misused during recent years.

It seems that this correlation was first used by Schröter for a purely didactic purpose. The hierarchal subordination of the species to the genus suggested to him a similar subordination of the association to the formation, considered as a superior ecological unit.

According to Tansley (69), "it is impossible to define the concept of the plant association, just as it is impossible to define the concept of a species." We are absolutely of the same opinion, but we must recall briefly some of the secondary questions which have been raised in the controversy concerning the analogies between species and associations.

From the taxonomic point of view, the species is regarded as an abstraction by the great majority of scientists.<sup>13</sup> Does the same hold true for the association?

This question has been asked directly by Alechin (1) and answered by him in the negative through a logic which seems to put all of us more or less in the wrong. This applies particularly to Du Rietz, whose fluctuating opinion, according to his own confession, was scarcely formulated before 1925 (Cf. Du Rietz, 17), and who has definitely declared himself for the exclusively concrete character and for the aggregate concept of the association in the sense of Nichols (42).

On the other hand, according to the most recent American contributions, the *stand* of Christopherson (11), of Nichols (44) and of Gleason (31) becomes the exact equivalent of the *Bestand* or *Einzelbestand* of the ecologists of the Braun-Blanquet school. "We consider every normally developed separate appearance of a definite association as an association-individual (local stand)," wrote Braun-Blanquet in 1921 (5).

The major importance of the stand as the concrete exemplification of the abstract association became clearly evident when Braun-

<sup>13</sup> For the contrary opinion, see Du Rietz (19). But when we say 'the tiger is cruel,' we are speaking in the abstract, and questionable logic can not change it.



Blanquet specified in 1921 (5) that each *Einzelbestand*, each individual of the association, could be used but once in the statistical determination of constancy. Kylin (37) devoted a dozen pages to the defense of the *association-individual*, the existence of which was to him the only reason for the distinction between frequency and constancy. According to Nordhagen (45) this idea is the absolutely indispensable basis for statistics of vegetation, since it concerns natural objects which are especially appropriate for the study of dispersion, homogeneity, and similar features.

Realizing that, according to the very exact definition of Braun-Blanquet, to deny the existence of individuals of an association is to deny the existence of the *Bestände* or stands, it appears to us useless to refuse such argument. We may merely recall that for Du Rietz himself (17) the idea of the individual of the association is considered a necessity for the partisans of the abstract association.

Then what remains to encourage polemics?

There remain all the pretexts of logic (?), all the linguistic chicanery introduced by Du Rietz (14) and continued with unflagging zeal against the term, against the expression, *association-individual*.

"Small pieces of any association have been termed 'individuals of the association,' a rather unhappy procedure and one now seldom used," wrote Lippmaa (39), who added, a few pages later, "We have seen that the idea of the individual of the association can not be maintained." The first phrase concerns a term, the second one an idea; these are by no means the same thing!

Lippmaa does not seem to have grasped exactly the thought of Vierhapper (75), who based his opposition on the literal interpretation of the term *individual*, an opposition therefore purely linguistic. In the ordinary anthropocentric language, this term evidently connotes a sort of constitutional unity, as realized for example in the body of a vertebrate, the dismemberment of which would lead to the actual loss of the individual personality.

Even Lüdi (41), one of the most brilliant representatives of the Zürich school, allowed himself to be influenced by the argument of Vierhapper. Although a firm defender of the *Bestand*, he condemned the term *association-individual* "not for reasons of logic, but because the term 'individual' awakens too easily the



impression of an indivisible thing, corresponding to an individual living thing."

When I proposed in 1912 (48) the term *individu d'association* (originated at the suggestion of Schröter in 1902), I had no other object than to enable the field ecologist to express his results in the French language as logically as the taxonomist expresses the results of his field study. The same argument was used later by Kylin (37) in favor of the *Bestand*, and Nichols (43) wrote at the same time "As will be apparent presently, the *stand* holds very much the same relation to the association \* \* that the individual plant, in the field of taxonomic botany, holds to the species."

In other words, and with philosophical reservation that "any comparison between the association and the species is purely in the nature of an analogy" (Nichols, 43), we may admit that the plant association, an abstract unit like the species, is represented, again like the species, by individuals (*individus d'association*, *Bestände*, *stands*), large or small in area and more or less scattered over the surface of the earth.

#### DIAGNOSIS OF ASSOCIATIONS

All those who during the past thirty-five years have followed the development of plant ecology can not have forgotten the deep impression created by the publication of Schröter's memoir "Vorschläge für eine Nomenklatur der Formationslehre," included in the botanical section of the *Monographie du lac Constance* (68). Under Schröter's vigorous impulse, synecology has impressed itself on the science of central Europe as a collective term, embracing all phases of the study of vegetation. It was his synecological concept, embodied in the definition of the plant association to which we have referred in the first lines of this article, which was presented, without much success, to be sure, to the consideration of the section of phytogeography at the Congress of Brussels in 1910.

There is one feature in this definition which has persisted since the beginning of the century and which has never ceased to hold good under the most diverse conditions: *the association should be characterized by its floristic elements*. One can even say that the search for the floristic solution of the problems of vegetation, regarded as groups of plants, is in some respects the very object of plant sociology.

The first solution which led to some fertile results is that of Brockmann-Jerosch (10), for it is he who for the first time advocated and practised the method of segregating the species of a vegetational group into three categories, the constants, the accessories, and the accidentals. He thus utilized a statistical selection with results which serve far better to characterize a group than a simple list of species. Only the methodical use of such principles of subordination appears to us to be capable of directing the search toward criteria which are truly indicative of and in conformity with the natural conditions and with the interdependence of the species; capable, in a word, of yielding essential and indispensable criteria for the rational diagnosis of plant associations.

Such, in our opinion, was also the chief value of the method introduced and gradually developed by Braun-Blanquet from 1913 to 1918. This places the chief stress on fidelity, a sociological idea, rather than on dominance or constancy, which are purely plastic or demographic ideas in the sense of Harper (33). It first attracted attention under the name of the floristic method, which Braun-Blanquet himself had given it, but it better deserved from the outset the name floristic-ecological method. Indeed, prejudice, as persistent as it is inaccurate, has often given it a strictly systematic character. But it appears from the words of Braun-Blanquet himself (3) that, although the method is based solely on floristic composition, it requires none the less an appreciation of the importance of the relation of the species to each other and to their environment which in itself is the only justification for separating these elements.

Then appear Du Rietz and his colleagues (22), whose laws maintain that all the past must be swept away in favor of the Upsala technique, which alone is wholly accurate and trustworthy. We have already mentioned the present attitude of Du Rietz toward this method of work, the abandonment of which—we might almost say the condemnation—coincides with a radical change in the style of his descriptions. The initiative of the Upsalians has nevertheless had an undeniable influence on the development of our science in continental Europe.

Before proceeding with the analysis, it is interesting to note the almost total lack of any noticeable reaction on the Anglo-American

scientific mentality. Since then as well as before, the great majority of ecologists of the English language have remained serenely faithful to the idea of dominants, in the sense of Clements, Tansley, and Nichols,<sup>14</sup> as the necessary and sufficient factors in the recognition, naming, and description of associations. Far from being misled or diverted by European trifles, they have continued to advance with admirable discipline along the path blazed by Cowles and by Clements, their eyes fixed on the splendid goal of positive knowledge and philosophical divination where shine, in letters of gold, the three magical words *succession*, *development*, *change*.<sup>15</sup>

Before closing these parenthetical remarks, it may be useful to recall that Du Rietz in 1930 (18), in his characterization of the sociation and other units in his system, gave a major rôle to dominants, which he defined as follows: "A dominant is a species which, either alone or together with one or several other species of about the same size, constitutes the bulk of the vegetation of its layer." The idea of stratification, more or less secondary in the definition of Tansley and Chipp, here assumes the rôle of a decisive systematic factor.

One is not surprised to find echoes of this idea in the most recent work of Katz (35) and of Lippmaa (40), but differing from each other in every respect.

Even more radical than Du Rietz himself, Katz claims that associations should be characterized only by the dominant species in each layer of the society. He defines a dominant species as "one which has the greatest dominance or the highest coverage in the association" (35). Floristic composition and stability, as well as physiognomy and site, should be eliminated, as useless or misleading, from the diagnosis of the association, which Katz accepts as the fundamental unit of plant sociology, but only in a very reduced size, corresponding to the sociation of Du Rietz (18).

According to Lippmaa (40), the separation of associations is possible only through the floristic composition as the point of de-

<sup>14</sup> A sense which is essentially physiognomic and plastic: "In all closed vegetation, i.e., where the ground is fully occupied by plants, certain species dominate the community. They are most commonly those which grow tallest and are most numerous in the upper layer or stratum of the vegetation" (73; see also 70, pp. 243, 246). "Certain plants are dominant, in the sense that they determine the physiognomy of the association" (44).

<sup>15</sup> The historical order. The logical order may be *change*, *succession*, *development*.

parture, and as practicable only in a stabilized vegetation in equilibrium with its environment. The technique recommended by Lippmaa for the study of associations in the field is that of Braun-Blanquet, but the Esthonian author exalts the importance of stratification more than any of his predecessors. For him each layer corresponds to a distinct association; as many the layers, so many the associations, and these layer-associations are for him the fundamental units of vegetation. In our opinion this process of systematic dismemberment of the association is likely to lead to a pulverization still more excessive than that of Du Rietz and Katz.<sup>16</sup>

But to return to our subject.

The idea of letting minimum area and minimum number of species play a part in the recognition of plant association was first formulated by Braun-Blanquet (2), whose most recent statement reads "A community of plants requires for its normal development a minimum area and also upon this area a minimum number of plant species." This idea of a similar relation between an area (minimum area) and a group of species qualitatively defined (the constants) as a necessary requirement for the diagnosis or definition of the association first appeared in the didactic memoir of Du Rietz and his colleagues in 1920 (22). Since that time the most striking result of their activity is that they have imposed on all of us the necessity of including statistical research in our general analyses of plant associations, thereby adding the quadrat method and the graphic expression of its results to the construction of a really accurate description.

Now the smallest amount of space which can be truly characteristic of a society is one which has a single individual of the rarest species, together with a mass of individuals of all other species of the society, the relative number and distance of the individuals of each species being determined by their respective density in the society. In practice, the size of the minimum area can be read directly from a graph showing the relation of species to area, or from the *species number : area* curve, reading the area as the abscissa of the point where the curve tends to approximate a straight line. In order to secure maximum accuracy in this com-

<sup>16</sup> Lippmaa foresees (40) that it will be necessary to distinguish two or three associations in a single "synusia of the second degree," in the sense of Gams (24).

putation, Lippmaa recommends that all accidental species should be neglected.

The actual number of species in this minimum area is in itself of only secondary interest. The essential value of its species, according to Braun-Blanquet (6), is that they represent a characteristic combination (an expression of E. Schmid, 67), comprising a limited number of species which appear in the area because of their high coefficients of fidelity and presence.

The importance of species of a high degree of presence is evident from the quantitative point of view, since the dominant species belong in this group. As to the characteristic species, in the sense of Braun-Blanquet, belonging to the three highest classes of fidelity, their qualitative importance is still greater from a sociological viewpoint, since they are often indicators of the ecology, development, or classification of the society.

Another stage was thus reached, whose opportuneness has stood the test of time.

But a beautiful, although essentially static, concept of the association was no longer sufficient against the irresistible wave of opinion which, originating in America, had already captured the British thought and aroused a lively interest in the dynamic and genetic viewpoint in several scientific centers of continental Europe.

On the other hand, the thorough study of the soil was brought into the lime-light at the same time through the results of research in biological applications of physical chemistry (46, 64, etc.) and in the genesis of soils by Glinka (32) and other Russian pedologists. As Pallmann and Haffner (47) justly say, "Soil, climate and vegetation form a solid *unit*. The properties of the soil are reflected in the characteristics of the vegetation which it produces, and that in turn reacts strongly on the formation of soil, while climate gives special features to both of them. Research in plant sociology is scarcely to be considered today unless it takes into account the environmental conditions of soil and climate." And Tansley writes: "The more detailed elucidation of the relation of soil evolution to the development of vegetation is the most important co-operative task of the immediate future for pedologists and ecologists."

To this program, which is now before us, several anticipatory answers have already been given during the past ten years. We

would particularly call attention to that of Braun-Blanquet and Jenny (9), based upon study of the alpine meadows in the Swiss National Park, since it deals especially with the parallelism between soil evolution and vegetational development and since it demonstrates in a general way the course of this double evolution over a large part of central Europe.

"In a work carried out in cooperation with a soil chemist (H. Jenny), we insisted, in 1926, on the fact that there exists a close and reciprocal relation between the natural development of the vegetation and the evolution of the soil. This relation, controlled primarily by the general climate of the region, discloses for central and northwestern Europe a progressive acidification of the soil, continuing to the final stage of soil maturity. Parallel with this acidification, the pioneer basiphile or weakly acidophile societies are succeeded by increasingly acidophile associations. The terminal stage, to which the succession tends, is purely acidophile. This evolution is not reversible; it may be considered a natural law.

"To the great climatic soil provinces (pedoclimax) there correspond climatic climaxes of vegetation." (Braun-Blanquet, 7.)

In other words, this evolutionary synthesis leads to the more significant idea, the vegetational climax on the mature soil, a symbol of perfect equilibrium and a criterion of stability, if not of permanence, as long as the present climate endures. If climatic climaxes were reached over the whole world, universal stability would result; this is not the case for many reasons. If it is true that "every association represents a more or less stable and enduring stage in the successional series" (Lüdi, 41), then the general characteristic of the present world is, in the brilliant phrase of Cooper (13), "the universality of change."

#### SUMMARY

1. The association as defined by Braun-Blanquet is the fundamental unit of plant sociology.
2. The plant association is an abstract unit, represented in nature by *individus d'association*, *Bestände*, *stands*, large or small, and more or less scattered over the surface of the earth.
3. There is a close reciprocal relation between the natural evolution of vegetation and the evolution of the soil. This evolution,



controlled primarily by the general climate, leads finally to the vegetational climax on mature soil. Before that stage, each association represents a stage of fluctuating stability and duration.

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# THE BOTANICAL REVIEW

VOL. I

JULY, 1935

No. 7

## UNSTABLE GENES

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The rediscovery of Mendel's papers<sup>1</sup> marks the beginning of the modern science of genetics. Since in these papers a definite gene concept was formulated, the origin of that concept may properly be placed at the same time as that of genetics although the term gene was not introduced until several years later (45).

The gene, as the unit of heredity, has attracted the attention of investigators since the early stages of genetic research. In spite of great interest in the gene problem and the large amount of work done on it, no method for direct study of gene properties has as yet been discovered. The closest approach to this was made by the recent discovery that changes in genes may be induced experimentally by x-rays and related radiations.

All our knowledge concerning the gene, therefore, is based upon observations made on visible effects of gene activity, which we have good reason to believe are not the primary ones. Changes in genes are manifested in certain characters in the organism and these characters, therefore, make possible a study of the behavior and characteristics of the genes. Hence the study of gene changes as seen in visible effects upon the organism becomes the most important method for study of the nature of the gene. This review will be devoted to results obtained in such studies and particularly to studies on unstable genes.

*Descriptions of two typical cases determined by unstable genes.* As an illustration of the behavior of unstable gene characters I shall give a short description of a simple case involving the variegated-rose of *Delphinium* (24) and another involving unstable miniature-3 character of *Drosophila virilis* (17). These cases are

<sup>1</sup> These two papers by the Austrian monk, Gregor Mendel, were published in 1866 and 1870, respectively, but did not receive much recognition until 1901 when the first of them was translated and published in English.—Editors.

selected as examples because they are simple and will be referred to again.

Flowers of the rose-variegated race of *Delphinium* show purple spots on rose background. These spots are interpreted as due to changes in the rose gene from rose into its purple allele.<sup>2</sup> Each of the spots, therefore, registers one such change which occurred sometime during development of the flower. If the change occurs early in development, the cell with the changed gene will have an opportunity to divide many times and, therefore, to produce a large spot; if the change occurs late in development, a small spot will be produced. Thus, size of spots indicates the time in ontogeny when the change occurred and number of spots shows frequency of changes. From seeds of a self-pollinated variegated plant, a few purple plants are obtained in addition to variegated offspring. These purple plants are the result of a change of the gene for rose into the gene for purple affecting the germ-cells. In *Delphinium*, offspring of seeds from such branches does not differ from offspring of seeds from variegated branches since color of flowers and germ-cells are located in two different tissues which separate very early in ontogeny, even before the change in the gene affecting a whole branch occurs. Flower color of *Delphinium* is present in only one epidermal layer of cells whereas germ-cells develop from sub-epidermal tissues. In some other color variegations, however, such as in variegated pericarp of maize (30), variegated flowers of *Antirrhinum* (64), in chlorophyll variegations and unstable morphological characters the germ-cells are affected by a change which covers a large enough area to include the region in which the flowers are located. In these cases, apparently, the germ-cells and the tissues showing unstable characteristics are derived from the same layer of cells; the gametes on the reverted branches are affected by the change since these gametes originate from the same tissue as the cells which show up the change in the phenotype.<sup>3</sup>

In its behavior, the unstable miniature character of *Drosophila*

<sup>2</sup> An *allelomorph* (allel) is one of two dissimilar factors which on account of their corresponding positions in corresponding chromosomes are subject to alternative inheritance (Darlington).—Editors.

<sup>3</sup> *Phenotype* refers to the external appearance produced by the reaction of an organism of a given *genotype* with a given environment. *Genotype* is the kind of type or the hereditary properties of an organism. (Darlington).—Editors.

*virilis* is similar to the variegated-rose character of *Delphinium*. A portion of the offspring of the parents carrying unstable miniature-3 gene is miniature, a portion of them has mosaic wings consisting of wild-type and of miniature tissues, and a few of them have wild-type wings. In this case the miniature gene is reverting to its wild-type allele. Wild-type regions on mosaic wings, therefore, are results of reversions which occurred during development of the wing, and the wild-type flies are reversions which affect the germ-cells.

*Direction of changes in unstable genes.* The important characteristics of unstable genes are that the prevalent changes in these genes go in one direction only and that these changes, in a great majority of cases, occur from a recessive<sup>4</sup> to a dominant allele which, in all but one known case, may be considered the wild-type allele of that unstable gene. For example, the gene for unstable white pericarp of maize changes into its dominant red allele (30), the gene for rose and lavender of *Delphinium* into the purple alleles (24), for dwarf of *Portulaca* into the wild-type (8), genes for contracted, cream, delicate, dotted, flecked, lobless, miniature, pine-inebustant, pupy, purple, speckled, wrinkled, yellow-inconstant and yellowy of *Pharbitis* all change into their respective wild-type alleles (44).

The case of the unstable willow-leaf of *Pharbitis* (43, 44) is the only one known to me wherein the gene changes into a dominant allele which is not the wild-type allele. As shown by Imai, the willow-leaf gene is unstable both in somatic and in germinal tissues and changes into the maple-leaf allele which is dominant to willow-leaf but recessive to the wild-type.

Another possible exception to what seems to be a general rule among unstable genes is the *crispa* case of *Antirrhinum* (7). *Crispa* is a dominant character, lethal when homozygous,<sup>5</sup> showing as dried out lesions especially on the edges of leaves. Baur found

<sup>4</sup> For every character of a sexually produced organism a gene or factor is ordinarily contributed by each parent. If each gene of such a pair is different from the other, as red and white rather than red and red, and if only one of them is visibly expressed, that one is said to be *dominant* over the other which is then *recessive*.—Editors.

<sup>5</sup> When both factors contributed by both parents affect a certain character in the progeny exactly alike, as red color from each parent, the offspring is said to be *homozygous* for that character. If the inherited genes or factors are unlike, as red and white, the offspring is *heterozygous*.—Editors.

that a strain, which had been tested in large numbers for three generations, produced about two per cent. *crispa* plants. He concluded that the wild-type allele of the *crispa* locus of that strain frequently changes into the *crispa* allele, that it is in an unstable condition. In this case, however, the possibility is not excluded that the *crispa* character is formed by some other mechanism than a gene mutation. Only one out of several such mechanisms will be mentioned here. If the strain throwing *crispa* should contain two partially overlapping inversions,<sup>6</sup> then a crossing-over<sup>7</sup> within inverted regions would produce a deficiency<sup>8</sup> which may readily show up as a dominant character and which almost certainly would be lethal when homozygous. The frequency with which such cross-overs would occur would be determined by various factors. It is probable that it would be low and that it could readily fulfill the two per cent. requirement. The *crispa* case, therefore, if included among unstable gene cases at all, should be included with reservations.

Changes in unstable genes usually go in one direction only, from a recessive into a dominant allele. This dominant allele is ordinarily as stable as any stable gene may be expected to be. Whether or not the change is reversible would be shown by the pattern formed by mosaic tissue. A reversible change in the case of a color character, for example, would give a double pattern, changes from light to dark showing as dark spots on the light background and changes in the opposite direction showing as light spots upon dark spots. Only one case of this type has been described so far (2). In several instances it has been claimed that gene changes occur in both directions (62, 48) but a careful analysis of the evidence presented makes these claims at least questionable. A close approach to a reversible change was described by Emerson (31) who observed that some of the solid red cobs of maize originating from variegated seeds had a few variegated seeds. That, however, was not a general characteristic of red cobs but was limited to a certain strain of maize. A good case of a reversible change has been described by Anderson and Ter Louw (2) in mosaic pericarp of

<sup>6</sup> An *inversion* is a reversal of the linear sequence of genes in one segment of a chromosome relative to an adjoining segment (Darlington).—Editors.

<sup>7</sup> *Crossing-over* is an interchange of corresponding segments of homologous chromosomes.—Editors.

<sup>8</sup> A *deficiency* is the loss of a segment of a chromosome.—Editors.



maize where the pattern produced indicates that the changes occur in both directions, from white to red and from red to white.

It is not easy to determine whether or not an unstable gene changes into its stable recessive allele because of great variability in frequency with which changes in unstable genes usually occur. To distinguish a constant allele from one changing with low frequency, a very large number of observations would be essential. Evidence available at present indicates that if such changes occur at all they occur with much lower frequency than changes to the dominant allele.

In addition to changes from the unstable recessive to the stable dominant, another type of change is evident among unstable genes, *viz.*, changes to various alleles differing from each other in the rate with which they change. It is probable that these changes are fairly common although they are not easy to detect because other factors cause rate of change to vary. The best evidence of changes from one unstable allele into another is available in the case of the unstable miniature-3 gene of *Drosophila virilis* (19, 21). Three miniature alleles are known in the miniature-3 series, *viz.*, miniature-alpha which is unstable both in germinal and in somatic tissues, miniature-gamma which is unstable in somatic tissues only and miniature-beta which is stable as far as changes into the wild-type are concerned. Miniature-alpha and gamma revert frequently into the wild-type and in addition to this, alpha rarely changes into gamma or beta, gamma rarely changes into alpha or beta, and beta rarely changes into alpha or gamma. Similar changes from one unstable allele into another were observed in maize (31) where pericarp variegation of the calico type, which affects somatic tissue and germ-cells, changes into dark crown variegation affecting somatic tissues only; in *Pharbitis* (44) where the unstable dusky gene produces several unstable alleles; and in *Polystichum* (4) where about six unstable alleles for chlorophyll variegation are known. Rate of change from one unstable allele into another is low when compared with the rate with which unstable alleles change into wild-type.

*Effect of various factors on rate of change in unstable genes.* It is known that the rate with which unstable genes change into the wild-type is influenced by various internal factors. Outstanding among these are various modifying genes. Three genes are

known, for example, which greatly increase rate of change in somatic cells of miniature-3 alpha and gamma of *Drosophila virilis* (22) and one gene is known which increases that rate in the germ-cells of miniature-3 alpha (23). Emerson (32) showed that modifying genes are responsible for increase of mutability of variegated pericarp of maize in crosses, *viz.*, when the unstable gene is in a heterozygous condition. A similar condition is probably responsible for such an increase in mutability as observed in *Mirabilis* (13). A decrease in rate of change of the globifera gene of *Antirrhinum* (41) was observed when this gene was heterozygous with its chlorantha allele and when it was compared with the rate of change observed in homozygous globifera or in the globifera/nicotianoides combination. Kihara (47) described a similar occurrence in *Celosia* where instability of the gene for the anthocyan color was decreased when it was combined with its stable allele.

Rate of change of certain unstable genes is affected by various ontogenetic factors. Evidence indicates that in *Delphinium* the unstable rose gene reverts to purple with constant frequency throughout all stages of ontogeny, but the unstable lavender gene reverts with high frequency in the early embryo and in late stages of flower development and it is either constant or reverts with low frequency at other stages of ontogeny (24). Anderson and Eyster (1) found that rate of change in variegated pericarp of maize increases toward the end of development of seeds. In *Pharbitis* Imai (44) found that yellow-inconstant-1 and flecked revert with a high rate early and late in ontogeny and with a low rate at other stages, while yellow-inconstant-2 reverts with low frequency at late stages and with high frequency at other times. As already mentioned, miniature-alpha of *Drosophila virilis* (21) is unstable both in somatic and in germinal tissues and miniature-gamma is unstable only in somatic cells. As far as is known at present, the reddish-alpha of *virilis* has the most restricted period of instability, reverting only at a maturation division of heterozygous females (20).

Several other factors affect rate of change of unstable genes. It is known that age reduces frequency of reversions in reddish-alpha of *Drosophila virilis* (20). It has been observed also that sex affects rate of change of the miniature-gamma gene, the rate in males being about twice as high as in females (26).

From the previous discussion it is evident that unstable genes are sensitive to various internal factors. Very few tests have been made to determine the effect of external factors on these genes. Eyster (34) reported in variegated pericarp of maize a significant difference in rate of change between material grown in a warm and that grown in a cool climate, the cool conditions producing an increased rate. Demerec (25, 28), however, found that neither a ten degree centigrade difference in temperature nor x-ray radiation significantly affected rate of change of the unstable miniature-3 gene of *Drosophila virilis*.

*Characters determined by unstable genes.* All types of characteristics are represented among those which are determined by unstable genes. As may be expected, characters showing color variegations are most frequently observed since they are easily detected. Many of them have also been preserved by breeders of ornamental plants and thus made available for study.

The variety of characteristics determined by unstable genes is best illustrated in the following list which is not intended to be complete. Various plant and flower color variegations are described in *Antirrhinum* (64, 5), *Celosia* (62), *Dahlia* (50), *Delphinium* (64, 24), *Hordeum* (58), *Impatiens* (39, 46), *Lathyrus*, (55), *Mirabilis* (14), *Miosotis* (10), *Nigella* (63) and *Zea* (30, 40); chlorophyll variegations are described in *Antirrhinum* (5, 49), *Barbarea* (3), *Capsella* (15), ferns (4), *Mirabilis* (14), *Nigella* (63), *Pisum* (38), *Plantago* (42) and *Viola* (12); of the morphological characters a description was made of globifera in *Antirrhinum* having small petals and only female flowers (5); phantastica and graminifolia in *Antirrhinum*, both having narrow leaves (6, 56); large grains in *Oryza* (61); contorta with whirled leaves in *Plantago* (42); ramosa with branched inflorescence in *Plantago* (64); dwarf plants in *Portulaca* (8); and of the physiological characters a sterility factor was described in *Antirrhinum* (5) and several such factors in *Oryza* (60, 52, 48). In *Petunia* an unstable gene was described which affects both color and size of flowers at the same time (57). In *Pharbitis* seventeen unstable genes are known affecting flower color, chlorophyll and various morphological characteristics (44). In *Drosophila virilis* three unstable characters are known, one affecting color of the body, one color of eyes and another size of wings (16, 17, 18).

*Occurrence of unstable genes in various organisms.* According to my list, there are at least 63 cases of unstable genes described among plants. In some plants numerous cases have been described; as many as 17 in *Pharbitis* and 7 in *Antirrhinum*; in others, relatively few are known as in maize where only two cases are known, one of which is still unpublished. This suggests that unstable genes may occur with different frequencies in different plants. As, however, there are many factors which can influence detection of an unstable gene, this difference is by no means conclusive.

Among animals only a few cases of unstable genes have been described. Three cases in *Drosophila virilis* (16, 17, 18) are unquestionably of that type. In *Drosophila melanogaster*, however, which is genetically much better known than *virilis*, only one character has been described as determined by an unstable gene (54). Patterson (53) reported a miniature wing character of *melanogaster* as being unstable, but the character was lost before it was fully investigated. Since at least ten times as many mutations have been observed in *melanogaster* as in *virilis*, difference in the number of unstable genes found in the two species may be due to specific differences between them.

As a possible case of an unstable gene, Castle (9) described repeated occurrence of a mosaic pattern in a strain of rabbits. Ferwerda (37) also described an eye character in *Tenebrio* which frequently gives spotted eyed and normal eyed individuals and which may be due to instability of the gene.

*Hypothesis.* DeVries (64) was the first to describe breeding experiments involving unstable genes. He recognized both somatic and germinal mutations and called them atavisms, *i.e.*, reversions to an ancestral form. Correns (14) realized that in homozygous variegation changes occur from a homozygous to a heterozygous condition. He visualized this change as due to the presence of an inactive gene for self-color among a gene or genes for variegation, which inactive gene becomes, under certain conditions, active, thus producing a change in the genotype.<sup>3</sup>

Emerson (30) was the first to interpret the behavior of variegations as being due to changes in the genes. For variegated pericarp of maize he assumed that red patches are the result of changes of the gene for variegation into its red allele. This interpretation

is generally accepted today. It agrees with all known facts. Recently, however, the cause of several variegated characteristics (51, 11) was traced to abnormal chromosome behavior and the suggestion was made (59) that the behavior of unstable genes might be interpreted in a similar manner, *i.e.*, they may be small duplications<sup>9</sup> and the appearance of the new form may be due, not to a change in a gene, but to a segregation of genes already present. The main opposition to such an interpretation lies in the fact that in all cases attributed to unstable genes, the change goes from the recessive to the dominant allele. If both recessive and dominant alleles were present in the unstable form, an improbable assumption would be essential, namely, that a dominant allele in combination with two recessives does not show its dominant effect. The analysis made with unstable genes of *Drosophila virilis* (17, 18, 20), where behavior of chromosomes was followed through a number of markers, indicates that no gross chromosomal abnormality is responsible for the changes observed. In case of reddish the cause for changes was traced down to within a region of only 2.8 cross-over units,<sup>10</sup> in which region it is known that the reddish gene is located. At present, Emerson's explanation is by far the most probable. A positive test on whether or not unstable genes are connected with duplications is now possible for *Drosophila* characters through use of the salivary chromosome method. These tests will be made as soon as the salivary chromosome structure of *virilis* is better known.

Correns (15), describing chlorophyll variegation of *Capsella*, again considers the theory of variegation. He looks upon changes as too frequent to be called mutations. He thinks of them rather as a disease of the gene, the varying degrees of disease being expressed as lighter or darker variegation. According to Correns, a gene which determines variegation may be pictured as a large molecule which consists of many identical atoms. The number of atoms is variable; it can increase or decrease. To each number of atoms in the molecule, corresponds a definite ratio of green to white tissue on the plant. Change in number of atoms can occur at any cell division. Only two stages are constant, *viz.*, the form

<sup>9</sup> A *duplication* is the occurrence of one segment of a chromosome twice in the same complement. (Darlington).—Editors.

<sup>10</sup> A *cross-over unit* is a one per-cent frequency of interchange between a pair of linked genes.—Editors.

with the maximum number and the one with the minimum number. The first combination determines green and the second white cells in case of a chlorophyll variegation. It is evident that Correns considered variegations as caused by changes in the genes although he did not call these changes mutations.

At the Toronto meeting of the American Association for the Advancement of Science (1920) E. G. Anderson suggested that unstable genes are composed of two kinds of particles, one responsible for the recessive type and one for the dominant type, and that changes observed in these genes are due to assortment of these particles within a gene. The gene responsible for variegated pericarp of maize, for example, would be composed of two kinds of particles, one kind determining red color and the other white color. Whenever the number of red particles is in excess of the threshold, color of the cell would be red, and when it is below the threshold, color would be white. The gene for stable red would have red particles only and the gene for stable white, white particles only. This suggestion has been elaborated and developed as an hypothesis by Eyster (33, 35, 36) who gave the name *genomeres* to particles which are supposed to be independent components of a gene. The first impression given by the genomere hypothesis is that it is simple and can explain not only the behavior of unstable genes but also the behavior of other genes as intimated by Eyster. When a close analysis of various cases is made, however, it becomes evident that in its simple form the hypothesis is applicable in only very few instances. To explain other cases, additional assumptions are essential, and if the whole field is to be covered, these assumptions become so numerous and involved as to make the whole hypothesis highly improbable. If it is assumed that an unstable gene is composed of two kinds of genomeres, that they divide at each gene division and segregate at random to daughter genes, it can be estimated from formulae developed by Dr. Sewall Wright (unpublished) that to obtain a constant rate of change of  $7^{-5}$  per cell generation observed in unstable rose of *Delphinium* (24), about 4000 genomeres would be required, but that with such a high number of components thousands of cell generations would be needed before the constant rate of change is approached. The observed behavior of the rose gene, therefore, cannot be explained by the genomere hypothesis



except, probably, by making involved assumptions. Similarly, observed facts that certain genes are stable at one stage of ontogeny and unstable at another, that certain genes change with different rates at various stages of ontogeny, and that various factors may influence rate of change can be explained by the genomere hypothesis only with the use of additional assumptions. If unstable genes were composed of genomeres, that should be true also of stable genes. In that case, it would be reasonable to assume that a mutation would begin with a change in a genomere, which process would be expected to produce unstable genes with high frequency. Ample evidence is available to show that this is not the case.

It has been suggested by Demerec (19, 24) that changes in unstable genes are caused by chemical processes rather than by mechanical assortment of particles within these genes. According to this view, an unstable gene is a chemically unstable entity changing into another definite form. Chemical reactions responsible for these changes may be influenced by various conditions of the gene environment. Thus, rate of change may vary at different stages of ontogeny, in different sexes and it may be influenced by certain other genes. It has been pointed out (27) that changes in unstable genes and changes in stable ones are chemical processes similar in nature, and also that there is no clear-cut difference between stable and unstable genes. Certain genes which are now called stable would be included in the unstable group if they were changing with the same rate in somatic cells where these changes could be easily detected, as they are changing in the germ-cells, where they are not easy to detect. At the same time a working hypothesis has been outlined, picturing a gene as a complex organic molecule and changes in genes as either slight rearrangements or changes in a molecular group of that molecule. An unstable gene, according to this concept, would have a molecular group in a chemically labile condition. Negative results obtained in temperature and x-ray treatments of unstable genes (25, 28) are interpreted as being due to the fact that the effects of these two treatments are so slight, in comparison with the natural rate of changes, that it is not possible to detect them.

Observations made on stable genes indicate that about seventy per cent. of the changes among them eliminate the gene (29). This elimination process is pictured as due to loss of reproductive



power of the gene because of the change which occurs. If changes in genes are considered as chemical reactions, that evidence would indicate that a gene may stand slight changes only; any extensive change destroys its power to reproduce and thus eliminates the gene.

The present day genetics concept visualizes the appearance of an organism as a result of an interaction of the whole set of genes the organism possesses and the environment in which it develops. A change in any of the genes, called a mutation, is liable to upset the balance of that system and show up on the organism as a character, usually as an abnormality, in some respect poorer than the wild-type. The appearance of a characteristic may be due to a change in a certain gene. That does not mean, however, that any single gene is entirely responsible for the development either of a particular character or a particular organ. Ample evidence is available to show that the final effect on the organism is produced through the interaction of the whole complement of genes, although certain of them may have greater influence on the expression of certain characteristics than others.

Studies with deficiencies, *viz.*, material where one or several genes are missing, show that the majority of deficiencies are lethal to the organism when present in a homozygous condition. This suggests that the presence of at least the majority of genes is essential in order that an organism may live. Moreover, the work with *Drosophila melanogaster* deficiencies (29) indicates that many of them are cell-lethal, *viz.*, that even a small group of cells located among normal tissues but containing a homozygous deficiency cannot exist. This suggests that genes are active in every cell and that, probably, the majority of them perform there a function highly important in the vital processes of the cell.

It has been pointed out (29) that a change in a gene which produces a visible effect (mutation), since it is detrimental to the organism, is not an effective means of evolution unless, if due to the change in the environment, the detrimental effect is either eliminated or changed into a beneficial effect. It is likely that more important for evolutionary processes are changes in genes which have a negligible effect on the organism. Accumulation of such changes in isolated groups of species may result in a significant difference between these groups. An addition of a new gene

(locus) which eventually would become so important as to act as a cell-lethal if deficient, would produce a fair degree of sterility between a group possessing such a locus and another one not having it and would, therefore, constitute an important step toward the formation of a new species.

It seems unnecessary in this review to discuss the well substantiated fact that genes are located in chromosomes, that they are there in a definite order which has a high degree of constancy. It may be mentioned only that it is not definitely known how much the appearance of a chromosome is determined by the genes it carries. There is evidence which indicates that there are chromosomes and chromosomal regions which carry very few, if any, genes but which in appearance and behavior are almost indistinguishable from the chromosomal regions containing genes. On the other hand, there is reason to believe that genes are even present in low organisms in which chromosomes are not detectable, and if that is true, the genes in these cases are independent of chromosomes. It seems justified to look upon a gene as a structure which was originally independent of a chromosome, but which, in early stages of evolutionary progress, either initiated formation of a chromosome by several of them forming a gene string or became associated with structures which later evolved into chromosomes.

In concluding this review, it may be pointed out that our knowledge about the gene is still in its infancy. Experimental methods (x-rays and salivary chromosomes) which became available to geneticists recently offer a means for more direct attack on the gene problem than was possible only a short time ago and give hopes for faster progress in the near future.

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# RESPIRATION

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## *Introduction*

By the term respiration is now understood the complex processes taking place in every living cell which involve a release of chemical energy utilisable in part for the building up of compounds of higher energy content and for other vital processes needing a supply of energy. While this release of energy is effected among bacteria in a variety of ways, in the vast majority of plants the normal respiratory process involves oxidation of carbohydrates and fats into carbon dioxide and water, oxygen being absorbed from the external medium to effect the oxidation, whence the whole complex of processes is described by the term "aerobic respiration." As is well known, a breakdown of carbohydrates takes place in plants when oxygen is excluded and a type of respiration, now generally known as "anaerobic," then occurs in which carbon dioxide is produced without a corresponding absorption of oxygen.

Investigations on respiration during the last five years have been concerned with both aerobic and anaerobic respiration in a variety of plant species and different plant organs in various stages of development. For most of these researches methods of measuring respiration have been employed which have for long been proved of service. These methods generally used involve determination of rate of evolution of carbon dioxide by the respiring tissue, the principle generally involved being the absorption in a standard solution of alkali of the carbon dioxide evolved. The solution is then titrated against standard acid, the decrease in alkalinity of the solution giving a measure of the amount of carbon dioxide absorbed. This method, in some modification of the form devised many years ago by Pettenkofer, is still regarded as the most reliable and most suitable for measurement of the respiratory activity of much plant material. An interesting modification of this method has been described by P. Emerson (11). The carbon dioxide is absorbed by barium hydroxide solution as in the usual procedure, but instead of titrating this with standard acid the amount of barium carbonate precipitated is determined by measur-

ing the height of the column of this precipitate in a tube under standard conditions.

The Pettenkofer method and its usual variants require a comparatively large amount of respiring material and experiments often have to be carried out over a few, and not infrequently, many hours, in order that the amount of carbon dioxide evolved and then absorbed by the alkaline solution shall be sufficient to alter the strength of the solution by a significant amount. Hence, where the respiration of small quantities of material over small intervals of time is to be measured the Pettenkofer method is inapplicable. For such work manometric methods, such as that involving the use of the Barcroft differential manometer and its variants, are frequently employed. Thus, in an investigation on the respiration of strawberry fruits, to which reference will be made later, A. R. Gerhart (16) used the Krajnik type of manometer. The principle involved is to allow the material to respire in a confined space and to absorb the carbon dioxide evolved with alkali. The gas in the space then loses the oxygen absorbed by the tissue and gains nothing from the latter. Consequently the change in volume under constant pressure is the volume of oxygen absorbed by the tissue. Obviously the smaller the respiration chamber the more sensitive the apparatus, so that the absorption of quite small amounts of oxygen can be determined.

But as the Pettenkofer method only gives a measure of carbon dioxide evolved, so the manometric methods only give a value for the oxygen absorbed. It is true that at the end of the experimental period with the manometric method the amount of carbon dioxide evolved by the alkali can be determined, but a series of observations on one sample of material can be made on oxygen intake only.

To overcome these limitations in respiration measurements inherent in the usual methods, W. Stiles and W. Leach (37) have developed the instrument known as the katharometer for the measurement of small amounts of respiration. The principles involved in the measurement of respiration by this instrument are as follows. When an electric current is passed through a wire it becomes heated, the temperature it acquires being in part determined by the thermal conductivity of the medium surrounding the wire, since the rate of removal of heat from the wire depends



on the thermal conductivity of the surroundings. Now the thermal conductivities of various gases are different, so that if the heated wire is contained in a vessel in which material is respiring, the replacement of oxygen by carbon dioxide will result in a change in the thermal conductivity of the gas in the vessel and hence in the temperature and electrical resistance of the wire. The replacement of air or oxygen by carbon dioxide has a very significant effect on the thermal conductivity of the gas and in the katharometer method of measuring respiration the output of carbon dioxide by respiring tissue is followed by determining the changes in electrical resistance of a heated wire in the respiration chamber. This method can be made very sensitive; indeed, by its means a change in carbon dioxide concentration of no more than .001 per cent. of the volume of the respiration chamber can be measured. Various improvements in the practice of the method were subsequently described by W. Leach (26). Thus by clock-work and photographic devices the apparatus can be made self-registering, while by the attachment of a manometer to the plant chamber changes in pressure can be measured and so data obtained for calculating not only the carbon dioxide evolved but the oxygen absorbed. Further, in this paper Leach also describes a device, based on the same principle as that used in the katharometer, by which changes in pressure can also be registered automatically. It is, however, an important limitation of the katharometer method that it is applicable in its present form only when the oxygen and carbon dioxide concentrations in the respiration chamber are changing. If the concentration of some other gas in the medium is changing as well, as, for example, if ethyl alcohol vapour should be given off by the respiring tissue, changes in thermal conductivity result from changes in both carbon dioxide and ethyl alcohol concentration. But if such a complication is not present the katharometer method affords a way of obtaining a continuous record of both carbon dioxide output and oxygen intake by small quantities of respiring material such as single germinating seeds, isolated small leaves and individual moss plants.

As regards the measurement of respiration, reference should be made to a paper by J. J. Willaman and W. R. Brown (47). These workers have devised a method for determining the amount of carbon dioxide dissolved in the sap of twigs and have applied

the method to work on apple twigs. The twigs are submerged in 95 per cent. alcohol in a closed vessel, the gas then removed under reduced pressure and the carbon dioxide absorbed in standard alkali and determined by subsequent titration of the alkali. The amount of carbon dioxide which can be removed from the twigs in this way is not inconsiderable, namely, about 150 to 250 mg. per kilo. of twigs at  $0^{\circ}$ , the amount declining with increase in temperature. Willaman and Brown consider that the presence of this dissolved carbon dioxide may explain three apparent phenomena of plant respiration. In the first place, when apple twigs are transferred from a lower to a higher temperature there results an increase in respiration which reaches a maximum and then falls subsequently to a level rate, the temporary rise being greater the lower the initial temperature. This temporary rise in respiration rate to a height above that characteristic of the temperature is attributed to lower solubility of carbon dioxide in the cell sap at the higher temperature with consequent release of the excess gas. Secondly, if tissue is kept in a closed vessel in which respiratory carbon dioxide accumulates and this carbon dioxide is removed and the respiration rate then measured, this rate also rises to a maximum and then falls to a level rate. The temporary rise is probably accounted for in part by release of carbon dioxide dissolved in the cell sap, since its concentration in the sap will be higher when there is a higher partial pressure of the gas outside the tissue than when the partial pressure is low. It is also suggested that higher acidity of the sap resulting from accumulation of carbon dioxide in it may also lead to a higher rate of carbon dioxide production. A third phenomenon connected with the solution of carbon dioxide in sap is the lower rate of carbon dioxide output by varieties of apples which possess greater winter hardiness. These varieties contain the lowest concentration of carbon dioxide in the sap but the cause of these relationships is not understood. In any case it may often be necessary, as Willaman and Brown point out, to distinguish between the measured rate of carbon dioxide output and the rate of carbon dioxide production by living tissue.

From some observations on the rate of respiration of potato tubers of different sizes in the case of three varieties, W. H. Michaels (32) concluded that the lenticels are the chief channels

of gaseous exchange between internal tissues and the surrounding air. While there is every reason to believe that this is so, it is difficult to understand how the data presented justify this conclusion, but the data themselves are not without interest. Thus, in the variety Early Ohio the respiration rates of large, medium and small tubers were found, respectively, to be .0296, .0312 and .0329 mg. carbon dioxide per sq. cm. of surface per hour, the corresponding respiration rates per lenticel being .0126, .0123 and .0075 mg. carbon dioxide per hour. The decrease in the rate of carbon dioxide production per lenticel in small tubers as compared with large ones is attributed to greater frequency of lenticels in the small tubers.

*The Influence of External Conditions on Respiratory Activity*

The chief external factors which might normally influence intensity of respiration in plant material are temperature, light, and the oxygen and carbon dioxide present in the atmosphere surrounding the tissue. A number of observations have been made during recent years also on the effect on respiration of various other substances when introduced into the external medium.

It is well known that the general effect of temperature on respiration over the range at which growth of most plants takes place, say from 5° to 30° C., is that the rate of respiration is increased from about twice to 2.5 times for a rise of 10° C. A number of recent researches, while indicating minor differences, have mainly served to extend the list of materials which obey the rule. Thus A. R. Gerhart (16) found that between 5° and 25° intensity of respiration of strawberry fruits is increased by almost exactly 2.5 times for a rise of 10°. Above 25° C., however, although the initial rate of respiration increases with increase in temperature up to 36.5° C., the initial rate is not maintained, the rate of respiration falling off more or less rapidly. The well-known "time factor" of F. F. Blackman is thus operative here. Gerhart's own opinion is that this time factor results from inability of oxygen to penetrate the cells rapidly enough to maintain the higher respiration rates. This further results in a certain amount of anaerobic respiration with the production of ethyl alcohol and other toxic substances which exert a deleterious effect on respiratory enzymes.

The same general relation between temperature and intensity of respiration was found, for the most part, by A. Hée (24) in seedlings of *Vicia faba*, *Zea mais* and *Lupinus*, in various leaves and in bulbs of *Allium*, as well as in the fungus *Sterigmatocystis nigra*. Similar results were obtained with seedlings of *Phaseolus aureus* by W. J. Crozier and A. E. Navez (9) and with seedlings of *Zea mais* and *Lupinus albus* by Pei-Sung Tang (40, 41, 42, 43). F. Kidd and C. West (25) found a similar effect of temperature on respiration in senescent apples. An interesting finding by Pei-Sung Tang is that oxygen absorption and carbon dioxide output are not affected to the same extent by temperature.

W. H. Michaels (31) concluded that change in temperature of the root of etiolated seedlings of *Phaseolus vulgaris* acted as a stimulus to the respiration intensity of the shoot. But fluctuations from hour to hour in respiration rate of the same root as measured by Michaels are so great that some doubt must be felt for the justification of the conclusion drawn. For example, respiration rate of roots grown at 15° C. during a number of successive hours varied from .77 to 9.48. Obviously any increase in respiration rate to be really significant would have to be very large, certainly much larger than those recorded which are of the same order as the fluctuations in respiration rate under constant temperature conditions.

After temperature, oxygen concentration as an external factor in respiration has attracted most attention during recent years. Until about 1930 the view was generally held that oxygen concentration was without effect on intensity of respiration unless the former was so low that anaerobic conditions were approached. However, in 1930, F. F. Blackman (7) announced that with apples in an atmosphere of oxygen and nitrogen, rate of respiration falls with increasing oxygen concentration until a minimum rate is reached in the neighbourhood of about 5 to 9 per cent. oxygen. Above this concentration the rate of respiration increases with increase in oxygen concentration until 100 per cent. oxygen is reached. In the same year W. B. Mack (28) published results of considerable research on the relation of temperature and oxygen concentration to respiration and growth of young wheat seedlings, respiration of these being measured at five different temperatures between 10° and 30° C., inclusive, and in twelve different oxygen concentrations ranging from .6 to 98.3 per cent. Here also the

relation of respiration rate to oxygen concentration was found not to be simple. For each temperature it was found that with increasing oxygen concentration from the lowest concentration used, the intensity of respiration also increased until a maximum was reached, the actual oxygen concentration at which this occurred depending on the temperature. Thus at 10° it occurred at from 6.3 to 9.8 per cent. and at 30° at from 10 to 16 per cent. With further increase in oxygen concentration there resulted a fall in respiration rate until a maximum was reached after which, with further increase in oxygen concentration, the rate again rose until a second maximum occurred at 90 or 95 per cent. oxygen. The rate of respiration at the highest oxygen concentration used, 98.3 per cent., was always lower than at 90 per cent. It seems clear that further investigation of the relation between respiration intensity and oxygen concentration is desirable.

The effect on respiration of various non-essential substances has been examined by various workers during the last five years and special attention has been directed to the effect of two toxic substances, hydrocyanic acid and ethylene. As regards the former, A. C. Shill (36) examined the effect on respiration of *Citrus* plants of a dosage of hydrocyanic acid about equal to that used in fumigation. It was found that the treatment brought about initially an increase in respiratory activity amounting to about 75 per cent. but that after about 35 hours the respiration rate returned to normal. An important contribution on the effect of hydrocyanic acid on respiration of potato tubers was published at about the same time by C. S. Hanes and J. Barker (22). They also found that with this material respiration rate at 15° C. increases as a result of exposure to an atmosphere containing hydrocyanic acid (.14 cc. to 1.45 cc. per litre) and then falls more slowly. In the highest concentration employed, however, the tissue suffers injury, but in the lower concentrations this is not the case. So long as the tissue is not injured the respiratory quotient,  $\text{CO}_2$  evolved:  $\text{O}_2$  absorbed, remains constant at about unity but in the high concentration the quotient rises to about 1.2.

The rise and fall in respiration rate accompany a corresponding rise and fall in sugar content of the tuber, and in moderate concentrations of cyanide it is thought that changes in respiration rate are due to corresponding changes in sugar concentration. These latter are themselves thought to be due to the direct effect of the

hydrocyanic acid on the starch-sugar relationships in the tuber. In the highest concentration of cyanide, when the respiration rate is falling from its maximum value, the ratio of respiration rate to sugar concentration falls, an effect attributed to inactivation of respiratory enzymes.

The effect of ethylene on respiration of wheat seedlings has been investigated by W. B. Mack and B. E. Livingston (29). A series of experiments was carried out similar to those described by the first named author to which reference has already been made, but with the addition of .1 per cent. ethylene to the experimental atmospheres. Various combinations of temperature and oxygen concentrations were employed as in the experiments without ethylene. It was found that this substance in the concentration employed has practically no effect on respiration at all temperatures when oxygen concentration is low (.6 per cent.) or high (75 per cent.; the highest concentration used in the experiments with ethylene). With oxygen concentrations of 20 and 50 per cent. also ethylene had little effect on respiration or retarded it somewhat, while in atmospheres containing 10 per cent. and 30 per cent. oxygen, ethylene accelerated carbon dioxide production. The complexity of the results very definitely indicates need for further investigation.

An increase in respiration rate of about 7.5 per cent. was observed by J. Green and A. H. Johnson (17) in bean leaves as a result of spraying with crude petroleum oils containing more than 16 per cent. sulphonatable residues, whereas the more refined oils brought about a decrease in respiration rate. The sulphonatable residue consists largely of unsaturated hydrocarbons and of sulphur, nitrogen and aromatic compounds. An increase in respiration rate of about 50 per cent. was also observed by M. P. Masure (30) when etiolated pea seedlings were irradiated with ultra-violet radiation.

#### *The Effect of Respiratory Substrate on Respiratory Activity*

The relation between concentration of sugar in respiring tissue and intensity of respiration has already been mentioned in dealing with the work of Hanes and Barker on effect of hydrocyanic acid on respiration. In two papers Barker (2, 3) has dealt with the



general question of relation between respiration of potatoes and concentration of sugars. The investigator found the problem complicated by a temperature effect which consists of a depression of respiratory activity as a result of exposure to low temperature. The depressing effect lasts for several weeks after a return to a higher temperature. It is regarded as the resultant of two processes, both affected by temperature but in opposite directions. These processes are the accumulation of the inhibitor, which is greater the lower the temperature, and the development of the inhibitory effect which is greater the higher the temperature and which has, indeed, a high temperature coefficient. Where the depressing effect is absent, sugar content has considerable effect on rate of respiration, as already indicated by the work of Hanes and Barker.

#### *Variations in Respiratory Activity During Development*

Change in respiration intensity of plants and plant organs in different stages of development has been recently investigated in a number of cases, particularly during germination and seedling development and during development and senescence of fruits.

Reference has already been made to Mack's observations (28) on respiration of wheat seedlings. Working with seedlings initially two days old he found over a period of about another two days a continuous rise in carbon dioxide output. In an investigation on the course of respiration of germinating seeds and seedlings of sweet pea (*Lathyrus odoratus*) Stiles and Leach (38) found the course of respiration depended largely on whether the seed coats were present or removed. If the seed coat is present and intact the course of respiration shows a series of phases. First, there is a fairly rapid increase in respiratory activity as the seeds absorb water. This is followed by the second phase, a period at which respiration rate remains constant, a state of affairs terminated by rupture of the seed coat which is followed by the third phase, a very rapid rise in respiration rate to a constant value characteristic of the fourth phase. This is finally succeeded by a decline in respiratory activity. If the testas are removed, the first phase of constant respiratory activity is eliminated and the respiration rate continually increases to the maximum of the fourth phase. The constant respiration rate of the second phase thus appears to be



related to the presence of the seed coat and may be conditioned by this latter limiting the diffusion of gases to and away from the respiring tissue, or may be related to inability of the seedling to grow in a confined space. The final falling off in respiratory activity appears to be due, either wholly or in part, to the conditions of experimentation, for the seedling is maintained in a closed chamber and reduction in transpiration may effect transference of respirable material from the cotyledons to the growing points, and so to a limitation of substrate at the places where respiration is most active.

A number of investigations of this kind have been carried out with fruit. Changes in respiratory activity of apples during senescence at different temperatures ( $2.5^{\circ}$ ,  $10^{\circ}$ ,  $22.5^{\circ}$ ) have been examined by F. Kidd and C. West (25). The course of respiration was similar at the three different temperatures, the respiratory activity rising to a maximum and then falling, the maximum value reached being about 1.5 times the initial value. This peak value is reached sooner the higher the temperature. Available evidence with regard to concentration of sugars in the tissues indicates that these changes in respiratory activity cannot be explained as due to changes in concentration of the sugar substrate of respiration, and it is suggested that the observed changes are related to some change of state in the colloidal matrix of the protoplasm. Such change might lead to a greater or less amount of effective enzymes or to a greater *effective* concentration of substrate, either by elution or by increase in permeability of the plasmatic membrane between protoplasm and vacuole.

The observations of Kidd and West were made on the English variety of apple, Bramley's Seedling. According to P. L. Harding (23), who also followed the respiratory activity of apples during storage at four temperatures ( $-1^{\circ}$ ,  $+2^{\circ}$ ,  $10^{\circ}$ ,  $15.5^{\circ}$ ), the respiration rate at each temperature increased during development and maturity. He worked, however, with the Grimes variety.

A paper on respiration of strawberries by A. R. Gerhart (16) has already been mentioned. E. L. Overholser, M. B. Hardy and H. D. Locklin (34) have also made a study of the respiration of this fruit. They also find that respiration is more rapid in mature than in immature fruits. Other aspects of the respiration of fruits will be dealt with later.

*The Respiratory Quotient*

It has for long been realised that a certain amount of insight into the respiratory process might be obtained from an examination of the relation between the amount of carbon dioxide evolved in respiration to the oxygen absorbed. The ratio of these two quantities is the respiratory quotient or respiratory coefficient, frequently now designated by the symbol  $R.Q.$  In particular a knowledge of its value might assist in deciding what materials were actually utilised as respiratory substrate. We have already noticed that in potato tubers concentration of sugar appears to have a definite determining effect on the rate of respiration, and if only sugar or some other carbohydrate is utilised, and if this is oxidised completely to carbon dioxide and water, and if there is no internal source of oxygen, and if all the carbon dioxide produced escapes from the tissues, then the respiratory quotient should be unity. If, on the other hand, fat is utilised, the respiratory quotient should be considerably below unity, actually in the neighbourhood of .7, since fats contain many fewer oxygen atoms than carbon atoms so that for the complete oxidation of a fat much more oxygen has to be absorbed relative to the amount of carbon in the substrate than for the complete oxidation of a sugar substrate.

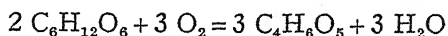
Germinating seeds provide excellent material for demonstrating the influence of substrate on the respiratory quotient. Thus Stiles and Leach (39) examined the change in value of the respiratory quotient during germination of seeds of a number of different species with different food reserves. For each species the quotient exhibits a continuous and characteristic change in value during germination. In *Zea mais*, where the reserve is chiefly starch but where a little fat is present, the quotient is initially about unity, falls to a minimum value of about .75 and then rises slowly towards unity. These changes in value of the quotient may be explained by supposing that at the beginning of germination a small quantity of sugar is present in the seed and this is at once used for respiration. As germination proceeds, fat becomes utilised and is either completely oxidised to carbon dioxide and water direct or, perhaps more probably, is first oxidised to sugar. In either case this would lead to a lowering of the respiratory quotient as actually observed. As the fat is removed, sugar derived from hydrolysis of starch by diastatic enzymes becomes the principal substrate and the respi-

ratory quotient therefore rises towards unity. Essentially similar behaviour was observed with other seeds in which the reserve material is chiefly starch, namely, *Lathyrus odoratus*, *Vicia faba* and *Pisum sativum*. In buckwheat (*Fagopyrum esculentum*) the initial fall in value of the respiratory quotient was not observed; it is either not present or is extremely rapid and the small quantity of fat in this seed appears to be utilised very rapidly for the quotient rises from its initial low value to near unity in a very few days.

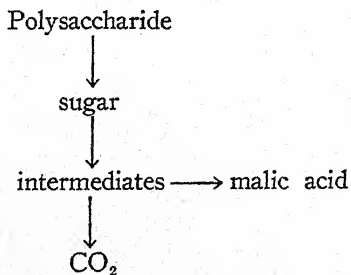
In seeds containing fats as the chief reserve there are minor irregularities, but, as is to be expected, the respiratory quotients are below unity. In *Ricinus communis* the respiratory quotient of germinating seeds falls regularly from about .85 to .5 in about 5 days, an observation in agreement with determinations made by A. I. Ermakoff and N. N. Iwanoff (12) and by J. R. Murlin (33). The latter even obtained values of the respiratory quotient of seedlings (with seed attached) as low as about .3. These low values can be readily explained only on the view that the fat is converted to sugar at a much greater rate than it, or sugar derived from it, is respired. With other fat-containing seeds, *Helianthus annuus* and *Cucurbita pepo*, there is generally first a rise in the quotient from about .75 to a maximum somewhat higher, after which there is a regular fall. The transitory rise may be due to utilisation of a little carbohydrate present in the seed along with the fat.

As shown by Aubert more than 40 years ago, the peculiar metabolism of succulent plants results in respiratory quotients in these plants of very varying magnitudes. The metabolism of succulents is still far from understood and in recent years fresh attacks have been made on the problems involved. T. A. Bennet-Clark (5), with a new type of apparatus (4), has examined the gaseous exchange in detached leaves of *Sedum praealtum*. When maintained in the dark the rate of carbon dioxide output falls, reaching a minimum about 6 hours after darkening; it then rises to a value which remains approximately constant for many days. At the same time the rate of oxygen intake rises to a maximum value, then falls to a minimum and then again rises until the rate of oxygen intake exceeds that of carbon dioxide output. As a result, the respiratory quotient for a succession of two-hour periods shows the following values: 1.46, .55, .17, .30, .78, .86, 1.40, 1.1. It will be recalled

that the generally accepted view of metabolism of succulent plants was that, in addition to some carbohydrates undergoing complete breakdown to carbon dioxide and water, some of the sugar suffers only partial oxidation with formation of malic acid according to the equation



Formation of a certain amount of malic acid in this way would thus involve absorption of oxygen for which no corresponding carbon dioxide is evolved and hence the measured respiratory quotient would be less than unity. Actually, when darkened, the acid content of leaves of *Sedum* rises until it reaches a maximum about 8 hours after the leaves are first darkened. For the next 8 to 12 hours the acid content falls, after which there is a further slight rise followed by a slow long-continued fall. It would appear then, that for the first 8 hours of darkness carbohydrate is being converted into malic acid and during this time the respiratory coefficient becomes considerably lower than unity. During the next period, when the acid is disappearing, the respiratory quotient rises and reaches values above unity and it is tempting to attribute this to utilisation of the malic acid as respiratory substrate. But the respiratory quotient, if the whole of the respiration took place in this way, would be 1.33, while actually still higher values were obtained; indeed, in one experiment a value as high as 2.05 was observed. The conclusion is therefore drawn that breakdown of carbohydrate is not directly through malic acid but through some other intermediate substance which requires less oxygen for its breakdown and the following scheme is suggested:



In this connection it would be interesting to know more exactly what takes place in other tissues containing much organic acid.

Unpublished observations in the reviewer's laboratory indicate that in apples, which contain much malic acid, the respiratory quotient may be well above 1.33, but there is at present no evidence that this is a general phenomenon for tissues containing much acid. In strawberries the available evidence presents contradictions for Gerhart (16) estimated the respiratory quotient to be about 1.2 while Overholser, Hardy and Locklin (34) found it to be about .9. Further observations on this question are obviously desirable and will no doubt provide valuable help towards solving the problem of the respiratory mechanism.

### *Anaerobic Respiration*

Insight into the respiratory mechanism has also been sought by the study of respiration in absence of oxygen and particularly the quantitative relationships between aerobic and anaerobic respiration. This had already been attempted with much success by F. F. Blackman and P. Parija (6, 8, 35) for the apple and as a result of their work a scheme was evolved to account for the relationship found experimentally. In brief, according to their scheme, respiration involves several stages. These are (1) hydrolysis of reserve carbohydrate to normal hexose, (2) activation of the hexose, (3) glycolysis of the activated hexose to intermediate products, (4) respiration in a restricted sense which takes a different course in absence and in presence respectively of oxygen. In absence of oxygen the products are alcohol and carbon dioxide, in presence of oxygen part of the intermediates is completely oxidised to carbon dioxide and water while part is built back into the system by a process of oxidative anabolism.

In more recent years descriptions of further work more or less on these lines have been published dealing mainly with seedlings and fruit. As regards the former, W. Leach and K. W. Dent (27) have examined the relation between aerobic and anaerobic respiration of germinating seeds in which the principal reserves are fats, while R. H. Dastur and R. M. Desai (10) have examined certain features of aerobic and anaerobic respiration in germinating rice seeds in which the reserve is chiefly carbohydrate.

In the 'fat' seedlings, those of *Ricinus communis*, *Helianthus annuus* and *Cucurbita pepo*, change in the surrounding medium from air to nitrogen brings about a rapid fall in respiration fol-

lowed by a continuous slow further falling off in respiration rate. On return to air after a 24-hour period in nitrogen the respiration rate rises rapidly and finally reaches normal for the respiration of the seedling in air. Leach and Dent think the behaviour described indicates that the respiratory substrate is not fat since the former appears to become exhausted if the seedlings are kept in nitrogen. The falling off of respiratory activity in nitrogen might be attributed to inactivation of enzymes concerned in respiration, but in this case it would scarcely be expected that the seedlings would resume so rapidly their normal respiratory activity when re-transferred to air.

It may be noted that the behaviour of these seedlings, like those of rice examined by Dastur and Desai, and of other seedlings containing much starch examined by Leach in researches as yet unpublished, is in marked contrast to that of apples examined by Blackman and Parija. In apple, respiration rate rises when the material is transferred to nitrogen to a value higher than the value for air. How far this behaviour is related to the stage of development or to chemical constituents of the respiring cells is not yet clear, but it may be mentioned that Gustafson (18) has found the same rise of respiration rate under anaerobic conditions in the fruit of the tomato. Moreover, the same worker has shown (19) that a number of cacti, including *Carnegiea gigantea*, *Echinocereus fendleri* and *Opuntia versicolor*, evolve as much, or nearly as much, carbon dioxide in absence as in presence of oxygen.

Information with regard to respiratory mechanism has also been sought by an endeavour to find evidence of intermediate products of respiration. If, as is usually supposed to be the case, aerobic and anaerobic respiration are connected, the connection is probably correctly indicated by Blackman's scheme mentioned above according to which, following the earlier views of Pfeffer and Kostytshev, intermediate products are converted either to alcohol and carbon dioxide in absence of oxygen or to carbon dioxide and water in its presence. Investigations on substances produced during anaerobiosis have been made in particular by M. Thomas and J. C. Fidler (13, 14, 15, 44, 45, 46) and by Gustafson (20, 21), the former working with apples, the latter with tomatoes and cacti. It is interesting and important to note that in these structures ethyl alcohol is found not only when oxygen



is absent but when it is present; indeed, in old apples Thomas and Fidler found that alcohol can accumulate even in an atmosphere of 100 per cent. oxygen. Acetaldehyde also appears to be present in all these tissues whether the conditions are aerobic or anaerobic.

Hence Thomas and Fidler are led to conclude that ethyl alcohol can be formed under aerobic as well as anaerobic conditions. In earlier work Thomas had distinguished between two types of what he called zymasis, that is, the splitting of carbohydrate with formation of ethyl alcohol and smaller quantities of acetaldehyde. He called these, respectively, anaerobic zymasis which takes place in absence of oxygen, and carbon dioxide zymasis which takes place in presence of oxygen when carbon dioxide is also present in relatively high concentration. In the former type there is a much higher percentage of acetaldehyde produced than in the latter. In his work on tomatoes Gustafson, however, was unable to find such a difference in the ratio of acetaldehyde to alcohol under the two sets of conditions.

In their later work, Thomas and Fidler conclude that in the apple senescence is accompanied by a change in the metabolism of the fruit whereby considerable amounts of ethyl alcohol and lesser amounts of acetaldehyde accumulate in the fruit kept in air. The higher the oxygen concentration the more the retardation of zymasis but as the fruit grows older the extinction point, that is, the concentration of oxygen required to suppress alcohol production, rises, so that in old apples it is not even reached in 100 per cent. oxygen.

If researches on these lines have not yet solved the problem of the intermediate products of respiration, they have brought to light new and important information and it cannot be doubted that continuance of work on these lines should yield valuable information on the nature of the respiratory mechanism.

*Relation of Rate of Carbon Dioxide Output to  
Rate of Loss of Substrate*

The last line of research to be considered here by which information regarding respiratory mechanism has been sought is the correlation of rate of carbon dioxide output with rate of disappearance of the substrate. This has been attempted by H. K. Archbold



and A. M. Barter (1) for apples and by Dastur and Desai (10) for rice seedlings. On F. F. Blackman's scheme, in aerobic respiration of apples, part of the substrate is rebuilt back into the system in the process termed oxidative anabolism and if the substance synthesized is not the substrate, there should be a corresponding divergence between the rate of carbon dioxide evolution and the rate of substrate loss. In apples Archbold and Barter actually found this was the case, the total amount of carbon in the sugar and malic acid lost being 17 to 30 per cent. greater than the carbon in the carbon dioxide evolved. In rice seedlings, however, Dastur and Desai conclude that for both aerobic and anaerobic respiration the carbon dioxide produced is greater than can be accounted for by the loss of carbohydrates. These workers suggest that the excess of carbon dioxide results from oxidation of plant acids produced in protein synthesis, but supporting evidence for this view has still to be obtained.

In conclusion it may be said that the work on respiration which has been carried out during the last five years, although containing nothing particularly spectacular, has materially advanced our knowledge both of the relationship of respiration to external and internal conditions and of the respiratory mechanism. If researches on the latter have succeeded in emphasizing the complexity and our ignorance of the process, they have added very materially to the data which will help in the end to the elucidation of the mechanism of this fundamental process of all living things.

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# THE BOTANICAL REVIEW

VOL. I

AUGUST, 1935

No. 8

## THE GENETICS OF BRYOPHYTES

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### *Heteroploidy*<sup>1</sup>

Genetic studies in this division having involved comparison of individuals of a given species differing in chromosome numbers, the occurrence and causation of such differences may first be noted.

It has long been known that protonemata may arise by proliferation from fragments of the immature sporophytes of certain Musci. References to earlier experiments and reports of additional results are given by Correns (22). Élie and Émile Marchal (41-43) induced "apospory" of this type in 19 mosses. In many of these species, the aposporously produced protonemata when grown under suitable conditions gave rise to leafy shoots (gametophores). In *Amblystegium serpens*, *Mnium hornum* and *Bryum capillare* aposporously developed gametophytes were found to possess the diploid chromosome numbers characteristic of the sporophytes from which they had arisen. Gametes produced by such diploid strains of dioecious species (*Bryum caespiticium*, *B. argenteum* and *Mnium hornum*) failed to function. Gametic union occurred, however, in aposporously produced diploid gametophytes of certain monoecious species (*Amblystegium serpens*, *A. subtile* and *Barbula muralis*). In the latter cases tetraploid sporophytes resulted (shown by cytological observation to be tetraploid in *Amblystegium serpens*); their spores were diploid and, in the 2 species of *Amblystegium*, gave rise to diploid gametophytes. Proliferation from tetraploid sporophytes of *A. serpens* gave rise to (presumably) tetraploid gametophytes. Tetraploid sporophytes of *Barbula muralis* produced aposporous protonemata which, however, bore no gametophores. The Marchals suggested that, since apospory may well occur in nature in consequence of injuries to

<sup>1</sup> Explanatory notes at end of article.

young sporophytes, it is not unlikely that diploid races may be thus produced from time to time. As yet, the chromosome numbers of too few mosses have been determined to show whether pairs of species, one of the pair having twice the chromosome complement of the other, are as frequent as this suggestion would lead us to expect.

Schweizer (49) obtained diploid gametophytes of *Splachnum sphaericum* by proliferation from sporophytes; triploid sporophytes by union of haploid antherozoids and diploid eggs; tetraploid sporophytes by union of diploid antherozoids and diploid eggs; and tetraploid gametophytes by regeneration from tetraploid sporophytes. Similar results were obtained by Bornhagen (19) in experiments with *Splachnum ampullaceum* as well as with *S. sphaericum*; and Schratz (46) secured by apospory diploid gametophytes of several species.

In a large number of mosses Wettstein (57-62, 64) similarly obtained diploid gametophytes. In some, including *Sphagnum* and Polytrichaceae, sporophytic proliferation never occurred. In certain species triploid sporophytes resulted from the union of diploid eggs with haploid antherozoids, and tetraploid sporophytes from union of diploid eggs with diploid antherozoids. Proliferation from triploid and tetraploid sporophytes, respectively, yielded triploid and tetraploid gametophytes. In *Funaria hygrometrica* fertilization within a triploid race gave rise to a presumably hexaploid sporophyte. In general, however, it was impossible in pure lines to secure either sporophytes or gametophytes with more than  $4n$  chromosomes, tetraploid gametophytes proving sterile. But by resorting to crosses between distinct species and even genera, Wettstein succeeded in obtaining plants of much higher valences. He describes gametophytes of hybrid origin with  $5n$ ,  $6n$ ,  $7n$ ,  $8n$ ,  $9n$  and  $17n$  chromosomes, and sporophytes with like numbers as well as one with  $32n$  chromosomes.

It should be added, however, that many strains of higher valence did not possess the exact chromosome complements indicated by the list just given. While meiosis ordinarily proceeds regularly in a diploid sporophyte, in sporophytes with higher chromosome numbers, even if, as in a tetraplont, an even number of chromosome complements is present, meiotic irregularities frequently result in the production of spores with varying chromosome numbers. Such

spores, if they germinate, give rise to correspondingly differing gametophytes. Wettstein thus obtained various aneuploid races, including hypohaplonts. Races and species differ greatly in the proportion of meiotic irregularities in polyploid sporophytes. In general, there is a tendency downward from higher chromosome complements toward the basic haploid number. This tendency is manifested in "vegetative regulation" in both gametophyte and sporophyte, as well as in the outcome of aberrant meioses.

The proportion of meiotic irregularities and hence the variety of chromosome combinations in spores and their offspring was increased by injection of capsules at the appropriate stage with solutions of chloral hydrate or of potassium nitrate. If such injections were made after the completion of the heterotypic division, the homoeotypic division was inhibited. The result then was the production of spore dyads instead of tetrads, each spore of such a dyad having the unreduced chromosome number. In this way, from normally diploid sporophytes, diploid spores and gametophytes of *Funaria* were obtained.

Another method used by Wettstein with *Funaria hygrometrica* and *Bryum caespitium* consisted in chilling or centrifuging protonemata, or treating them with ether or chloroform vapor or with chloral hydrate. An inhibition of cell division, as in Gerassimoff's classical experiments with *Spirogyra*, induced the appearance of diploid cells from which diploid protonemata and gametophores developed. From matings between such diplonts, and between them and haplonts, came triploid and tetraploid sporophytes; and from these by proliferation triploid and tetraploid gametophytes.

Attempts to induce apospory in Hepaticae by wounding developing sporophytes have thus far succeeded only with *Anthoceros*.<sup>2</sup> Lang (36) observed regeneration from such sporophytes in *A. laevis*. Later studies have been made of the thalli so produced in *A. laevis* and *A. Husnoti* by Schwarzenbach (48) and in *A. laevis* by Bornhagen (18). Chromosome numbers were not determined.

Heteroploidy caused in other ways appears, however, to be not infrequent among hepatics. Showalter (50-52) described a diploid

<sup>2</sup>Lorbeer (39) announces the induction of apospory in 52 species of hepatics. His detailed account has not yet appeared; apparently "apospory" in his experiments implies a failure of meiotic chromosome-pairing in spore mother cells.



gametophyte of *Pellia Neesiana*, found growing in nature and kept for some time in culture. Spores from a sporophyte borne by this plant produced diploid gametophytes. Heitz (31, 32) found races of *Pellia epiphylla* possessing 18 instead of the usual 9 chromosomes. On the basis of chromosomal characters, Jachimsky (34) concludes that one of Heitz's diploid races is of hybrid origin. Lorbeer (39), on the other hand, proposes a new species to include the diploid plants described by both Showalter and Heitz.

In several species and races of *Sphaerocarpos* whose spores normally remain attached in tetrads, the latter are occasionally replaced by dyads or more rarely by triads (12, 14, 37). While ordinarily of rare occurrence, in some matings of *S. Donnellii* dyads are relatively numerous (14). Their occurrence has been explained (39) by "a mitosis with avoidance of a reduction division." The most common type of dyad gives rise to diploid intersexual (but functionally female) offspring having one X and one Y chromosome<sup>3</sup> (12, 14, 37). Such a diploid gametophyte, mated with a haploid male, produces (presumably) triploid sporophytes whose spores in turn are ordinarily arranged in typical-appearing tetrads. Spores of one such tetrad gave rise to a haploid male gametophyte with 1 Y chromosome and a diploid intersexual gametophyte with 1 X and 1 Y (14). The occasional dyads produced by the triploid sporophytes consist probably of triploid spores; but none of these has yet germinated.

In the same species, one diploid male gametophyte with 2 Y chromosomes (14) and one diploid female with 2 X's (13) have appeared, both from spores of unknown pedigree. The diploid female, mated with a haploid male, gave rise through a triploid sporophyte to a considerable gametophytic progeny. The chromosomal constitution of some of these is under investigation.

Polyploid races of *Marchantia* are described by Burgeff (21) and Haupt (29). Diploid gametophytes arose from various crosses between races of *M. polymorpha*. Diploidy, it is suggested, may be explained by the observed occurrence of occasional irregularities in meiosis, sometimes leading to production of but 2 or 3 spores from a mother cell. Triplonts and tetraplonts occasionally appeared, in some instances coming from crosses between diplonts, but they grew slowly and died early. Among hyperhaplonts, one

<sup>3</sup> X and Y chromosomes are explained on page 274.

had 10 (instead of 9) chromosomes. The additional element was an autosome. Hyperhaplonts of another class have 1 or 2 extra sex chromosomes. *Marchantia "grisea"* presents a unique situation (30). A male race has the 9 chromosomes typical of several species of the genus; one monoecious race has 10, of which a very small "z" chromosome is eliminated in the formation of antheridia. Monoecious races of this species from other localities possess 2 to 5 small chromatic elements instead of a "z" chromosome.

Further indications of the occurrence of polyploidy appear in the literature. Counts of "ca.8" (31) and 18 (29) are given for *Marchantia planiloba*; of 8 (39) and of 18 (53) for *Riccia Gougetiana*; and in plants of *R. Donnellii* from a single collection, Siler (53) found, respectively, 8 and 16. Heitz (31) called attention to the fact that in a list of chromosome numbers of hepatics studied by him, 36 species have 8, 9 or 10 chromosomes, whereas 14 have numbers ranging about 16, 24 or 32.

In summary: a diploid gametophyte may arise by proliferation from a sporophyte (mosses, *Anthoceros*); through inhibition of cell division in a haploid protonema (*Funaria*, *Bryum*); through an inhibited meiosis, either spontaneous or due to the operation of a genetic factor (*Sphaerocarpos*), or caused by artificial means (*Funaria*).

A triploid sporophyte may be produced by union of a diploid and a haploid gamete; a tetraploid sporophyte by the union of 2 diploid gametes; and sporophytes of higher valences by the union of gametes with appropriate chromosomal endowment.

A triploid or tetraploid gametophyte or one of higher valence may arise (in mosses) by proliferation from a sporophyte of corresponding constitution.

Aneuploid gametophytes may come from spores produced by irregular meioses, induced either by treatment or (especially in polyploid mosses) by unknown causes; or in consequence of irregular mitoses in gametophyte or sporophyte.

Union of gametes from aneuploid gametophytes may give rise to aneuploid sporophytes.

#### *Sexual Characters*

Genetic studies of bryophytes have dealt more extensively with sexual than with what may roughly be termed vegetative charac-

ters. It is convenient, therefore, to consider this portion of the field separately. Such consideration does not imply that the genetic bases of sexual characters are of fundamentally different nature from those of other characters.

Bryophytes, as found in nature, fall into two sharply contrasted classes: some species are dioecious, each individual gametophyte being either purely male or purely female; others are monoecious (in the broadest sense of the term), each gametophyte being capable of producing gametes of both sexes.

Compilations based on taxonomic lists (24, 39) indicate that somewhat more than half the species of mosses and of liverworts are dioecious. The apparent proportional prevalence of dioecism is likely, however, to be reduced as critical studies are made of individual species. For example, *Funaria hygrometrica* and *Preissia commutata*, among others once classed as dioecious, have proved monoecious on fuller study.

Strict dioecism implies that the entire clone descended from a single spore, including all individual plants derived by vegetative means from the original thallus, shoot or protonema, is of the same sex. The extensive cultures and experiments necessary to demonstrate the existence or non-existence of such absolute dioecism have as yet covered but a small minority of species. They begin with Noll's experiments on *Marchantia polymorpha* (17, 47) and, for the Musci, with the work of the Marchals (40). For a fairly considerable number of both hepatics and mosses it can now be said that a given spore transmits to its gametophytic progeny a single sexual potentiality whose expression can not be reversed by any known environmental change. For another considerable class it is clear that each spore transmits both female and male potentialities.

The first clue to the mechanism of sex-determination in plants was supplied by *Sphaerocarpos*. In most species and races of this genus, the 4 spores derived from a single mother cell are permanently adherent. Upon germination, 2 of the spores of such a tetrad (in at least 3 species of this genus) regularly produce female, 2 male gametophytes (2, 26, 37). The gametophytes of *S. Donnellii* are distinguished by the presence in the female of a very large X chromosome and in the male of a very small Y chromosome (1, 2). The X and the Y are separated in the division of

the spore-mother-cell nucleus, an X passing to each of 2 spores of the tetrad, a Y to each of the other 2. Sex-determination, therefore, is effected in meiosis.

A similar chromosomal mechanism, the X being larger than the Y, has now been reported in about 20 dioecious hepatics and in 4 dioecious mosses. Recent lists are given by Lorbeer (39) and Tinney (55). Regarding 2 of these species (*Riccardia pinguis* and *Riccia Curtisii*) reports are conflicting, possibly because of confusion in nomenclature. In 2 other species, *Tesselina pyramidata* and *Lunularia cruciata*, the Y is found to be larger than the X; and in 2 *Frullanias* there is said to be no Y, the female having one more chromosome than the male.

In some other dioecious bryophytes, investigation has failed to disclose the presence of recognizable sex chromosomes. The distribution of sex potentialities indicates, however, that in such species sex is determined in essentially the same manner as in *Sphaerocarpos*.

Aposporously produced diploid gametophytes of dioecious mosses—having both maternal and paternal chromosome complements derived directly from the sporophyte—are regularly monoecious. This was first shown by the Marchals (41) for *Bryum caespitium*, *B. argenteum* and *Mnium hornum*. Intersexual organs are sometimes borne by these aposporous diplonts, of a nature similar to those occasionally observed (20, 33) in normally monoecious mosses. In the Marchal's diploid clones, antheridia first appeared and were consistently produced more abundantly than were archegonia. In Schweizer's *Splachnum* diplonts, approximately the reverse condition prevailed.

Although both sex organs are present, the gametes of monoecious diplonts derived by proliferation from the sporophytes of dioecious species seem to be generally non-functional (42), differing from the gametes of corresponding diplonts from normally monoecious species. A few sporophytes were, however, exceptionally produced (58) in diploid cultures of *Bryum caespitium*. *Splachnum sphaericum* also constitutes an exception; consequently, Schweizer secured triploid and tetraploid sporophytes of this species, and from the latter by proliferation tetraploid gametophytes. These gametophytes produced antheridia; they were not kept in

culture long enough to determine whether or not archegonia also might appear.

Diplonts derived by inhibition of cell division from haploid protonemata of *Bryum caespiticium* are regularly fertile (57, 58). Diplonts so obtained from female protonemata are female; those from male protonemata, male. A diploid female fertilized by a haploid male produced a sporophyte which by proliferation gave triploid gametophytes of whose chromosome complements 2 were maternal and 1 paternal. Such gametophytes were more strongly female than diploid gametophytes produced by proliferation, hence possessing but 1 maternal and 1 paternal complement; the former produced antheridia and archegonia in the ratio of 1.37:1; the latter, in the ratio of 5:1. Tetraploid gametophytes proliferated from tetraploid sporophytes possessed 2 maternal and 2 paternal chromosome complements. These in turn were more strongly male; the ratio of antheridia to archegonia was 13.3:1.

The rule that diploid gametophytes of dioecious species are monoecious does not necessarily hold for aneuploid, especially for hypodiploid derivatives. In the Marchals' (43) cultures of *Phascum cuspidatum*, aposporous diploid gametophytes were almost completely devoid of sex organs; such gametophytes were variable among themselves and very different in appearance from normal haplonts. These, with 3 exceptional "monstrous" clones of *Splachnum sphaericum* described by Schweizer (49), may well have been aneuploid races. The aberrant clones of *Splachnum* were, respectively: female, fertile; female, sterile; male, probably sterile—in contrast with the majority of diplonts which were bisexual and fertile. Bornhagen (19) secured only diplonts of the fertile female type. Wettstein (58) obtained aneuploid unisexual gametophytes by regeneration from sporophytes of *Bryum caespiticium*.

Among hepatics, the sexual conditions in heteroplonts are in harmony with those in mosses possessing corresponding chromosome complements. Showalter's (50–52) diploid *Pellia Neesiana*, with apparently 1 maternal and 1 paternal complement, was monoecious, bearing some aberrant structures which may have been intersexual organs. Its gametophytic offspring, resulting from self-fertilization, were likewise diploid and monoecious.

In *Sphaerocarpos Donnellii* (12–14), a diplont with 2 X's is female; one with 2 Y's, male; and one with an X and a Y is inter-

sexual as is shown by the presence of organs intermediate in structure between antheridia and archegonia, although it is female in appearance and some of its eggs are capable of fertilization.

Diplonts resulting from crosses between dioecious haploid races of *Marchantia polymorpha* were female if they had 2 X chromosomes and male if possessing 2 Y's. Haupt's apparently diploid *M. planiloba* was female; however, she cites an observation by Bergdolt (16) of bisexual plants of this species. It is suggested that diploid races occur, some with 2 maternal, some with 1 maternal and 1 paternal chromosome complement. Haupt's hyperhaploids with 2 X chromosomes are female and fertile. Those with 1 X and 1 or 2 Y's are female in appearance but sterile. The case of her *M. "grisea"* is not entirely clear. The male race has 9 chromosomes, including presumably 1 Y; the monoecious races have in addition either 1 "z" element or 2 to 5 small chromatic bodies.

In general harmony with the facts already cited is Heitz's (31) observation that of 33 hepatics with chromosome numbers from 8 to 10, 24 are dioecious and only 9 monoecious; whereas of 14 with multiples or approximate multiples of these numbers, 13 are monoecious and 1 is doubtfully dioecious. The suggestion is that monoecious diploid forms have arisen in nature from dioecious haploid species.

Proponents of various current hypotheses of the mechanism of sex-inheritance have sought to apply each his own favorite theory to the determination of sex in general. This attempt involves the assumption that sex must be similarly controlled in all sexually differentiated organisms. In this regard, if the assumption is sound, sexual characters differ from all others; for it is well recognized that the same phenotypic character may be conditioned in different species, often even in the same species, by the interaction of very different gene complexes. Indeed, a similar variance with respect to sexuality is shown by the synthesis of dioecious races of corn (27, 35), sexual differentiation being determined in the respective races by different genes. Comparable results have been obtained with *Lebistes* (68).

For bryophytes the mechanism of sex-determination seems, on the basis of present knowledge, relatively simple. There is no evidence that in any dioecious bryophyte each haploid individual pos-



sesses the potentialities of both sexes, as postulated by Correns (24) and Goldschmidt (28). Nor is there evidence that factors for sexual differentiation are, in bryophytes, borne on the autosomes, as appears to be the case in *Drosophila*.

In a dioecious bryophyte, sex depends solely, so far as is now known, upon the sex chromosome or chromosomes present. A haploid gametophyte is female if it has an X chromosome; male if it has a Y. A diploid gametophyte is female with 2 X's; male with 2 Y's; hermaphroditic with an X and a Y. Hermaphroditism is expressed in *Sphaerocarpos* by the appearance of intersexual organs; in this genus the female tendency introduced by the X chromosome is almost but not quite dominant to the male tendency supplied by the Y. The Y plays a positive part in sex-determination in bryophytes, very different from the neutral or near-neutral rôle of the Y in *Drosophila*.

An explanation of sex-determination in bryophytes, so far as such an explanation can now appropriately be formulated (12), includes the assumption of a factor or factor-complex borne on the X chromosome which endows the gametophyte possessing it with female potentialities; and of a factor or factor-complex borne on the Y chromosome which enables the gametophyte possessing it to develop male characteristics. It must be supposed, of course, that the development of sexual as of other characters involves the interaction of the whole genic system; the determinative factors for sex are effective only in such interaction. However, the distinctive characteristics of either sex can not appear in the absence of factors borne by the appropriate sex chromosome. With an X or X's and no Y, only female potentialities are present; with a Y or Y's and no X, only male potentialities; with both an X and a Y, both female and male potentialities exist and may find expression.

The problem of sex-determination does not exist in relation to those bryophytes which in the haploid condition are monoecious. In such species each gametophyte possesses the genetic bases for the production of the characters of both sexes. It is impossible at present to connect such genetic bases with specific chromosomes. As Heitz has suggested, it is not unlikely that some (or many) monoecious bryophytes are derived from diploid races of originally dioecious species; on this supposition, male and female potentialities depend upon factors borne on separate chromosomes both



of which are transmitted regularly to all offspring. Proliferation from any part of a monoecious gametophyte, even from a sex organ, produces a monoecious plant (23, 58). Diploid gametophytes and those of higher valences derived by sporophytic proliferation from monoecious species are, as would be expected, monoecious.

However, it is evident that there must be in monoecious bryophytes, especially in mosses, many genetic factors which influence the position, distribution and order of appearance of sex organs. Such factors find expression in the varied specific arrangements of these organs described respectively as autoicous, synoicous, paroicous, etc. Nothing can be stated beyond the inference that such factors exist. Schellenberg (44) and Bornhagen (19) have shown something regarding the influence of external conditions upon the expression of factors of this class.

#### *Vegetative Characters*

Comparative sizes of corresponding cells and organs of haploid and diploid moss gametophytes have been much studied; to some extent also those of higher polyploid races. In general, the larger the chromosome number the larger the cell. The ratio of cell volume in haplonts to that in corresponding organs of diplonts of the same species ranges about 1:2, varying characteristically, however, from species to species, and being affected also by external conditions (58, 60-62). The Marchals (42) reported a similar ratio as to nuclear size; later writers have found the results of nuclear measurements unsatisfactory. Plants of higher than diploid valence have usually larger cells; but increase in size does not so closely parallel increase in chromosome content. In most reported instances, cell volume increases more in proportion from  $2n$  to  $4n$  than from  $n$  to  $2n$ . Volumes of leaf cells in *Funaria hygrometrica* (58) gave the ratios ( $n$ ) 1: ( $2n$ ) 2.4: ( $3n$ ) 4.56: ( $4n$ ) 6.88. In a series of measurements in the same race made by another method (60), the corresponding ratios were 1:1.83:3.17:5.48—the increase here being in an almost exact geometrical ratio.

Differences in number or size of chloroplasts are sometimes correlated with differences in chromosome content. In diploid *Physcomytrium eurystomum* (46) the leaf cells have about 3 times as many chloroplasts as do corresponding cells of haplonts, individual

chloroplasts being about  $1\frac{1}{2}$  times as large. Ratios of chloroplast numbers per cell in *Amblystegium serpens* (58, 61, 62) are ( $n$ ) 1: ( $2n$ ) 1.8: ( $4n$ ) 4.86. No significant difference in size of chloroplasts was noted. In other species, chloroplasts of polyploid races are sometimes smaller than those of haplonts.

In general, dimensions of corresponding organs vary in the same direction as differences in chromosome number (42, 46). But the actual proportions of organ size to cell size vary greatly (58), since in different cases the number of cells in a particular organ of a diplont is equal to, greater than, or less than the number in the corresponding organ of a haplont.

Increase in size consequent upon increase in chromosome number often operates differently in different axes of the cell (58). Increase in length may be greater than increase in width, or *vice versa*. Resultant modifications in shape of constituent cells affect the forms of organs, leading in diplonts to forking of leaves and other marked variations. Such "abnormalities" are more evident in tetraplonts, and still more marked in aneuploid clones.

An increase in chromosome number produces a reduced osmotic value in gametophytic cells (15). An added chromosome complement from another species in consequence of hybridization (as *Funaria* chromosomes in *Physcomitrium* cytoplasm) has less effect than if the added nuclear substances belong to the same species. Addition of a second or third strange chromosome set has little osmotic effect.

The proportional number of sex organs commonly diminishes with an increase in chromosome complement. An extreme case of this nature is that of the diploid (or possibly aneuploid) gametophytes of *Phascum cuspidatum* (43), which were almost devoid of sex organs. These plants furnish a unique instance of variation in a diploid race by the production of structures, possibly gemmae, at the ends of leaves, which are unknown in haplonts of this species.

One plant derived from a spore of a tetraploid sporophyte of *Physcomitrium pyriforme* was hemiploid (45, 66, 67), having but 18 chromosomes whereas the ordinary haploid gametophytes of the species have 36. This plant, while smaller in all parts than the haploid parent form, grew well, was fully self-fertile and gave a constant progeny. By proliferation from its sporophytes arose a 36-chromosome ("diplohemiploid") race differing in various char-

acters from 36-chromosome *P. pyriforme*. Cell volumes of the hemiplont were to those of haploid (36-chromosome) *P. pyriforme* as 1 to 1.16; to those of the diplohemiplont as 1 to 2.62. The corresponding ratio of haploid to diploid *P. pyriforme* was 1:2.59. That is, the diplohemiplont with 36 chromosomes had cells nearly as large as those of the diploid (72-chromosome) race of the original species. The hemiplont, mated with another presumably hemiploid plant of similar origin, produced a sporophyte which by proliferation gave rise to a typical *P. pyriforme*. It is concluded that this species possesses 2 different sets of 18 chromosomes each, either of which is adequate to the development of a viable and constant race.

Relations of size and form among diploid, triploid and tetraploid sporophytes are of the order of those among gametophytes of corresponding valences. The number of sporophytes produced by a diploid clone (whose gametes are capable of union) is usually proportionally less than the number borne by a haplont (58). This difference is accounted for partly by the smaller number of sex organs, particularly of archegonia, and partly by the more sluggish movement of diploid antherozoids.

In *Anthoceros*, differently from mosses, the cells of (presumably) diploid thalli are smaller than those of a haplont (18, 48). The darker green color of a diplont results from the fact that, although its cells are smaller, the chloroplasts (usually 1 to a cell) are of about the same size as those of a haplont (48).

Lorbeer (38) finds the average volume of cells in thallus lobes of female *Sphaerocarpos* to be about 1.7 that in the male. This difference is approximately proportional to the difference between the chromosome content of the respective sexes which results chiefly from the great size of the X as compared with that of the Y chromosome. As was long ago pointed out, however (2), the much greater size of the female gametophyte and of all its parts at corresponding stages of development is due in the main to differences, not in cell size but in number of cells and consequently in rate of cell-growth and division. A spore with an X chromosome is reported also (39) to be larger than one with a Y.

Many references to the occurrence in nature of supposed hybrids between species of Musci, based upon the morphological characters of the plants in question, appear in the literature. (For lists

and résumés see 54 and 61.) No natural hepatic hybrids are reported. Successful hybridization experiments have involved races of *Funaria hygrometrica* and distinct species of Funariaceae (57-62, 64-66), races of *Sphaerocarpos Donnellii* and distinct species of *Sphaerocarpos* (3-11, 14), and races of *Marchantia polymorpha* (21).

In *Funaria hygrometrica*, 6 pairs of characters are distinguished which in interracial matings (57, 58, 60, 61, 64) behave as though determined each by a single pair of genes. Genes governing spore-size and rate of division of protonemal cells are completely linked<sup>4</sup> or, alternatively, the same gene influences both characters. A similar relation holds between leaf-form (a gametophytic character) and the form of seta and capsule (sporophytic). Other characters studied are form of paraphyses and capsule-color.  $F_1$  sporophytic characters are intermediate with indications of partial dominance, and are alike in reciprocal matings. Spores of  $F_1$  sporophytes fall into 2 approximately equal size-classes. Gametophytic progeny display the alternative parental characters, as expected, in a ratio of about 1:1. Differences in cell-size between races of this species are to be explained by the effects of several pairs of genes (56).

Combinations in triplonts and haplonts of varying proportions of genes determining either sporophytic or gametophytic characters show corresponding variation in dominance relations. In case one gene is present in double the quantity of its allele, nearly or quite complete dominance is manifest.

The following interspecific moss crosses have been successful (57-60, 64): *Funaria hygrometrica*  $\times$  *F. mediterranea* and reciprocal; *Physcomitrium eurystomum*  $\times$  *P. pyriforme* and reciprocal; *Funaria hygrometrica*  $\times$  *Entosthodon fascicularis* and reciprocal; *Funaria hygrometrica*  $\times$  *Physcomitrium pyriforme* and reciprocal; *Funaria hygrometrica*  $\times$  *Physcomitrium eurystomum* and reciprocal; *Physcomitrella patens*  $\times$  *Funaria hygrometrica*; *Physcomitrella patens*  $\times$  *Physcomitrium pyriforme*; *Physcomitrella patens*  $\times$  *Physcomitrium eurystomum* and reciprocal.

By combination of the method of securing polyploid gametophytes by sporophytic proliferation with that of interspecific cross-

<sup>4</sup>Later studies (25) indicate that rate of division in protonemata of *Funaria* and *Physcomitrium pyriforme* is affected by at least 2 gene-pairs.

ing, combinations were obtained of varying numbers of chromosome complexes from the respective species.

Sporophytes from reciprocal interspecific crosses differ in varying degrees. Their spores are largely, in some crosses entirely, non-viable. The gametophytic progeny, when obtainable, display a variety of combinations of parental characters. With increasing representation of the chromosome complement from one parental species (in crosses involving polyploidy), variations in dominance relations appear, as in corresponding intraspecific matings.

*Physcomitrium*  $\times$  *Funaria* (both haploid) yielded sporophytes showing chiefly dominance of *Physcomitrium* characters, but of the capsule color of *Funaria*. In consequence of irregularities, the gametophytes from spores produced by such sporophytes were variable. In normally developing, apparently haploid gametophytes, maternal characters strongly predominated; the majority were of purely maternal character. More or less diploid offspring showed a large proportion of paternal characters. Wettstein concluded that the *Funaria* antherozoid contributes no cytoplasm to the zygote; and that after chromosome reduction, spores containing only the *Funaria* chromosome complex in *Physcomitrium* cytoplasm are non-viable. In approximately diploid spores, a complete *Physcomitrium* chromosome complex is accompanied by a greater or less number of *Funaria* chromosomes which may carry dominant *Funaria* genes. The explanation is supported by the fact that sporophytes from *Physcomitrella*  $\times$  *Physcomitrium eurystomum* (and reciprocal) produce some spores which remain in tetrads. Of the spores of such a tetrad only 2 germinate, and these give rise to gametophytes purely or almost purely of maternal type.

From results of the various crosses in question, Wettstein (60, 63-65) concludes that the cytoplasm as well as the chromosomes plays an important genetic rôle. The cytoplasm of different races of *Funaria hygrometrica* is so similar that reciprocal crosses give like results and the reappearance of parental characters in the offspring is determined entirely by the genes. Cytoplasmic differences between distinct species of *Funaria* produce marked differences between the offspring of reciprocal crosses. Some characters of the gametophytic progeny are determined by the genes, the distribution being Mendelian; some are determined by the cytoplasm, the result being maternal inheritance; some, by the com-

bined influence of genes and cytoplasm. In crosses between *Physcomitrium* and *Funaria*, as has been seen, there is evidence of so great cytoplasmic difference that chromosomes of one parent alone are non-viable in the cytoplasm of the other parent. Finally, in still wider intergeneric crosses, cytoplasmic differences are so large that the proportion of sterility is very high and such spores as germinate produce plants of purely maternal type.

In crosses involving *Physcomitrium* and *Funaria* (66), carried on in various combinations through several generations, no evidence was obtained of an influence of the paternal chromosome complex upon the genetic nature of the cytoplasm.

The races of *Sphaerocarpos Donnellii* chiefly used in genetic studies have spores persistently adherent in tetrads, each tetrad resulting from the division of a spore mother cell. Of the gametophytic characters whose inheritance has been studied, polyclady (3, 5, 7, 10, 11, 69) has given the most clear-cut results. The character, which affects particularly the forms of antheridial and archegonial involucre, is inherited as though determined by a single gene. Sporophytes from a mating of non-polycladous  $\times$  polycladous produce tetrads, 2 of whose spores give rise to typical, 2 to polycladous gametophytes. Tetrads of such a sporophyte fall into the following classes: (1) those with 2 spores genetically female non-polycladous, 2 male polycladous (the parental combinations), 56 per cent; (2) those with 2 spores female polycladous, 2 male non-polycladous, 27 per cent; (3) those with 1 spore female polycladous, 1 female non-polycladous, 1 male polycladous, 1 male non-polycladous, 17 per cent. The preponderance of class (1) over class (2) indicates something like a linkage between sex and the polycladous character; but since a crossing over between the X and the Y chromosomes seems out of question, it has been suggested that there is in this case a tendency for certain chromosomes derived from each parent to pass to the same daughter nucleus in meiosis. The occurrence of class (3) shows that some segregation takes place in the second meiotic division; a result which would follow upon a crossing over involving one of each pair of sister chromatids of the chromosome pair bearing the differential genes for polyclady and non-polyclady. Female polycladous clones bear only very rare archegonia and have proved invariably sterile. Evidence has appeared (unpublished) of the oc-



currence of a modifying factor which increases somewhat the proportion of typical sex organs and involucre in polycladous clones of both sexes.

Two other characters, vegetative (9 and unpublished) and appendiculate (unpublished), are also apparently inherited in a unitary fashion; but the distinctions between typical plants and those genetically vegetative or appendiculate are not sufficiently sharp to make the results of their mating entirely clear.

Tuftedness (4, 10, 11), affecting, like polyclady though less strikingly, the forms of involucre, is a variable character. It appears in some branches of a clone others of whose branches are non-tufted. In different clones, very different degrees of tuftedness appear. A tufted branch in such a clone is more likely, as experiment shows, to give rise vegetatively to tufted than to non-tufted branches; a non-tufted branch is more likely to produce non-tufted than tufted branches. The inheritance of tuftedness is complicated; apparently it is due to a number of variously related factors.

A male "semi-sterile" clone (10, 11, 69), producing a very small proportion of antheridia, has given unexpected results. Mated with a typical female, it yields only typical offspring; with a tufted female, typical and tufted offspring. The semi-sterile character has never appeared in any progeny, although large families have been produced, and although in some cases all the spores of a tetrad germinated. The semi-sterile character seems not to be represented in the chromosomes of the semi-sterile clone, whose character nevertheless has remained constant during about 12 years of vegetative multiplication. Possibly, however, the explanation lies in an inability of the semi-sterile gene to function in a strange cytoplasm.

Similar, but limited and therefore less conclusive, results have come from matings of a "cupulate" male, a very distinctive clone which arose as a mutant (10, 11). The cupulate character has not reappeared in any offspring.

Another male mutant, a dwarf (unpublished), though bearing antheridia in abundance, has thus far proved completely sterile.

Matings between races of *S. Donnellii* having respectively separate spores and spores adherent in tetrads show that the characters of this pair are inherited only through the mother (6, 10). That



is, the genetic character of the mother determines whether the sporophytes it bears, whatever the inheritance of the father, shall produce spores with the one character or the other. The inheritance of either character of the pair, now traced through several successive generations, follows the descent of the X chromosome. It appears, therefore, that the character-distinction in question is sex-linked in the sense that the differential gene is borne by the X chromosome, although its expression appears in the asexual generation.

Hybrid sporophytes have been obtained from a few crosses between *S. texanus* ♀ and *S. Donnellii* ♂ (10, 14). The species are sharply distinguished by the spore markings. The spores of the hybrid sporophyte have at least the general and most distinctive characters of the maternal species. Apparently the character of spore-marking is inherited in the same manner as is that of spore-adherence or separation—following in descent the course of the X chromosome. It may be added that the American forms hitherto classed as *S. texanus* constitute a complex which includes at least 2 probably distinct species. All these forms, however, are readily distinguishable from *S. Donnellii*. The sporophytes obtained in these matings are the only interspecific hybrids yet reported in Hepaticae.

The mutation rate in *S. Donnellii*, on the basis of 1273 gametophytes of known ancestry, is, under greenhouse conditions, about 0.4 per cent (10, 11).

The occurrence of several mutations in *Marchantia polymorpha* has been briefly reported (21). Some of these are interpreted as indicating a possible method of origin of the characters of related genera. Successful matings were made involving 3 of the mutant characters; the results led to the assumption of the presence of latent factors in the haploid chromosome complex.

#### EXPLANATORY NOTES

Bryophytes include the *Hepaticae* or liverworts and the *Musci* or mosses. The life cycle of a liverwort or moss includes two phases: the *gametophyte* and the *sporophyte*. The gametophyte is the larger, longer-lived generation. In a liverwort, the gametophyte is either a flat, more or less ribbon-shaped branching plant, or a branching stem-like structure bearing leaves. The gametophyte of a moss begins as a branching filamentous *protonema*, from which arise leafy shoots. The sporophyte in all bryophytes is a relatively small plant, parasitic upon the gametophyte.

The gametophyte bears sex organs, *archegonia* and *antheridia*. If both these organs are produced on the same plant, the liverwort or moss is *monoecious*; if on different plants, it is *dioecious*. A gametophyte is *haploid*, since each of its cells contains one set ( $n$ ) of chromosomes. The union of an *egg*, within an archegonium, with an *antherozoid* (male gamete) from an antheridium, forms a zygote with  $2n$  chromosomes. From this arises a sporophyte, which is *diploid* because each of its cells has  $2n$  chromosomes.

The sporophyte, attached to the gametophyte, develops a *capsule* (as well as, in most species, a *stalk* and *foot*). Spores are formed within the capsule. In their formation two nuclear and two cell divisions occur, so that four spores are formed from each spore mother cell. These two nuclear divisions (*reduction* or *meiotic* divisions) differ from other nuclear divisions in the fact that they bring about a reduction of the chromosome number from  $2n$  to  $n$ . The first meiotic division, in which corresponding chromosomes pair and separate as wholes, is the *heterotypic* division. In consequence of these divisions, each spore has  $n$  chromosomes, and may develop into a haploid gametophyte.

The plants (gametophytes or sporophytes), according to the number of sets of chromosomes in each cell, are *haplonts* ( $n$ ), *diplonts* ( $2n$ ), *triponts* ( $3n$ ), *tetraplonts* ( $4n$ ), etc. Plants with valences higher than haploid (in the gametophyte) or diploid (in the sporophyte) are *polyploid*. Any plant having other than the typical haploid or diploid number is *heteroploid*. An *aneuploid* plant has an uneven multiple of the basic number ( $n$ ) of chromosomes.

*Autoicous*: having male and female organs (archegonia and antheridia) on the same plant but in separate clusters.

*Synoicous*: having male and female organs intermixed in the same cluster.

*Paroicous*: having male and female organs in the same cluster, but in distinct groups.

*Chromatids*: half-chromosomes resulting from longitudinal division, which later become daughter chromosomes.

*Crossing over*: the exchange, during meiotic prophase, of corresponding segments between the chromatids of paired chromosomes.

The *genotype* of an individual (plant or animal) is the sum total of its hereditary endowment. Its *phenotype* is its actual character, determined by the interaction of the genotype and the environment.

*Polycladous*: referring to a race of *Sphaerocarpos* marked among other things by frequent branching.

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## THE CYTOGENETICS OF MAIZE

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Cytogenetics may be defined as the correlation of cytologically observed conditions with genetic data.

Although genetic studies with *Zea mays* began little more than twenty years ago and cytogenetic investigations are of much more recent date, an impressive amount of information has been accumulated concerning the heredity of the maize plant. No other plant has been studied from the cytogenetic point of view so intensively as has maize, and the purpose of this article is to present some of the more pertinent facts which have been discovered.

Genetic characters in maize show great diversity of effects but can be roughly classified into three groups on the basis of the type of tissue affected: (1) sporophytic characters ( $2n$  tissue); (2) gametophytic characters ( $1n$  tissue); and (3) endosperm and aleurone characters ( $3n$  tissue).<sup>1</sup> A large majority of the known genes affect the sporophyte. Here are found a hundred or so genes for chlorophyll development alone; also genes for plant stature, plant color, modifications of male and female inflorescences, sterility, etc. Gametophytic genes are much fewer in number but among them are found genes for selective fertilization (due presumably to slower pollen tube growth or pollen germination), for pollen size, for ovule abortion and for type of carbohydrate in pollen grain and embryo sac. Endosperm and aleurone genes include those determining color and composition of endosperm and color of the aleurone layer. This classification of genes into those affecting the sporophyte, gametophyte and endosperm is an arbitrary one and does not imply that a given gene cannot affect more than one type of tissue. The waxy (*wx*) gene, for example, determines the nature of carbohydrate stored in both gametophytic and endosperm tissue.

Many genetic characters in maize represent some gross morphological modification in the normal structure of various parts of the plant. There are, however, some genes which affect chromosome behavior and cell division (5, 6, 7, 9, 10, 12). The asynaptic gene<sup>2</sup> (*as*), when present in a homozygous<sup>3</sup> condition, disturbs in



some way the normal pairing of chromosomes in early prophase so that most of the chromosomes are present as univalents<sup>2</sup> at diakinesis<sup>2</sup> and metaphase.<sup>2</sup> The sticky gene (*st*), as its name implies, causes the chromosomes to stick together so that at metaphase I there is a clumped mass of chromatin rather than 10 independent bivalent pairs. The sticky gene apparently increases frequency of non-disjunction,<sup>4</sup> gene mutation and produces translocations,<sup>5</sup> deficiencies<sup>6</sup> and chromosome fragments. The polymitotic gene (*po*) causes the haploid nuclei in each spore of the quartet of spores, formed at the end of the second meiotic division, to undergo a series of division-like figures followed by cytokinesis in which the chromosomes are segregated to the two poles without splitting equationally (lengthwise). There are two variable sterile genes (*va*<sub>1</sub>, *va*<sub>2</sub>) which tend to prevent occurrence of cytokinesis<sup>7</sup> in meiotic divisions and which cause an apparent tendency of the chromosomes in the microspores to undergo a precocious division. The effects of these genes suggest that not only development of the plant as a whole proceeds under the influence of genes but that the chromosomes themselves, which carry the genes, are under genic control.

The number of mutant genes in maize is about 400, the great majority of them being recessive to the normal or usual condition.

The diploid<sup>8</sup> or somatic number of chromosomes in *Zea* is twenty. The monoploid or gametic number is ten. All of these chromosomes are morphologically distinguishable. The morphology has been studied both in somatic and meiotic divisions but study of the meiotic prophase has given far more information concerning the detailed morphology. In the meiotic prophase the chromosomes are long slender threads many times longer than at somatic metaphase, and the different chromosomes can be recognized by their relative lengths, the positions of the non-stainable spindle fiber<sup>4</sup> attachment regions and the presence of deep staining knobs in specific positions on certain of the chromosomes. The number of knobs varies in different stocks but when a particular knob is present it is a constant feature of that chromosome and is inherited with the same precision as a gene. One of the chromosomes is especially conspicuous because it possesses a satellite and is always found in association with the nucleolus.<sup>9</sup> A deep staining somewhat reticulate body adjacent to the nucleolus on this chromosome is responsible for the orderly organization of the

nucleoli in the telophases<sup>4</sup> (48). The morphology of the ten chromosomes is shown diagrammatically in figure 1.

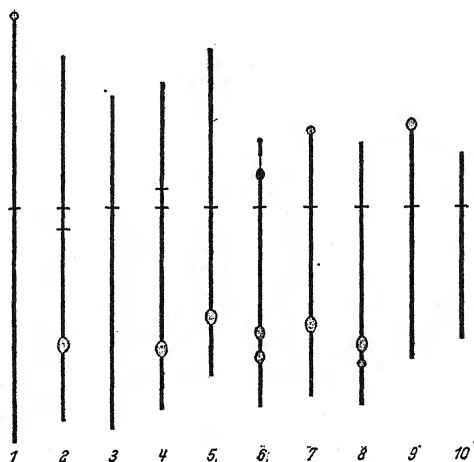


FIG. 1. Diagram of the ten chromosomes composing the monoploid complement of *Zea mays*. The positions of the spindle fiber attachment regions are indicated by cross lines. In chromosomes 2 and 4 two positions for this region have been found in the different cultures of maize. In chromosome 4 this difference in position has been correlated with an inversion. The positions of the knobs which are most likely to appear in the different cultures of maize are indicated. In any one culture a particular knob may or may not be present. (From McClintock, 1933.)

Since there are ten members of the monoploid<sup>1</sup> complement, all different in morphology, it is expected that the heritable characters in maize would fall into ten linkage groups which would show independence in inheritance with one another. Such is the case and figure 2 gives the ten linkage groups with the map distance (*i.e.*, cross-over value<sup>10</sup>) between the different linked genes. Only those genes whose approximate location in the genetic map is known are listed. About one half of the 400 known genes have been assigned to special linkage groups.

Not only has it been shown that the genetic characters in maize fall into ten linkage groups in correspondence with the number of chromosomes but each linkage group has been associated with a specific chromosome. The occurrence of a triploid<sup>1</sup> plant in 1925 (53, 42) was the starting point for the association of chromosomes and linkage groups. Theoretically the gametes produced by a triploid plant should have chromosome numbers ranging from ten

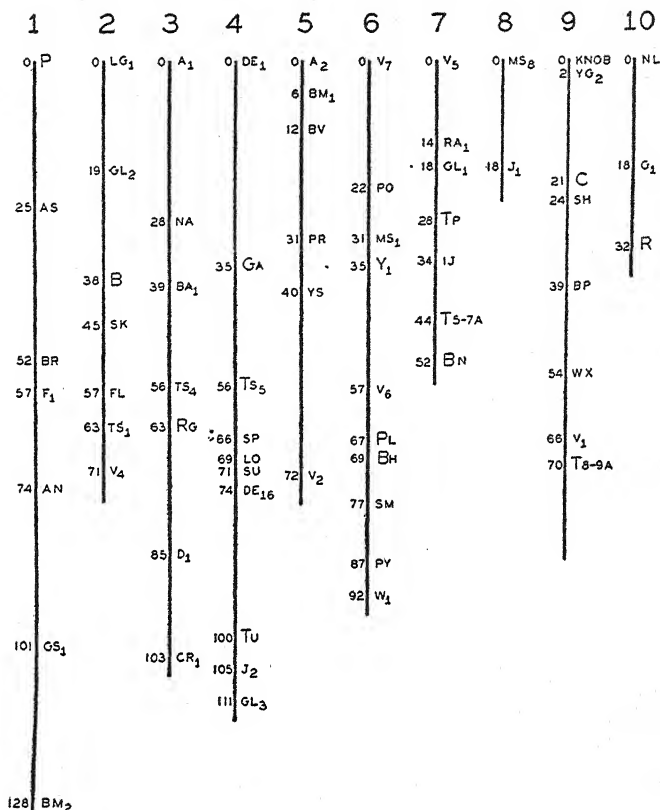


FIG. 2. The ten linkage groups in maize with the map distances (cross-over values) between the different linked genes. The genes comprising linkage group 1 are carried in chromosome 1 (see figure 1), the genes in linkage group 2 are carried in chromosome 2, etc. The lengths of the genetic maps of the different linkage groups do not agree with the lengths of their associated chromosomes because certain linkage groups have been more extensively studied than others. Only those genes whose loci are known with approximate certainty are shown in the genetic maps.

to twenty and the cross of triploid with a diploid should give individuals with chromosome numbers ranging from twenty to thirty. Such was the case and from these individuals  $2n + 1^{11}$  plants were isolated possessing different members of the set of 10 chromosomes as the extra chromosome. Cytological examination showed which member of the set of 10 was present in triplicate. These different trisomic<sup>12</sup> types were crossed with plants carrying genes in the ten

linkage groups.  $F_1$   $2n+1$  plants were either selfed or back-crossed to individuals homozygous for the recessive genes. When the progeny of the  $F_1$   $2n+1$  individuals gave 3:1 and 1:1 ratios, respectively, normal diploid ratios, it was proof that the linkage group being tested was not associated with the trisomic chromosome under consideration. When, however, the progeny of the  $F_1$   $2n+1$  individuals gave widely divergent ratios, or so-called trisomic ratios,<sup>13</sup> it was evidence that the particular linkage group being tested was associated with the particular trisomic being studied.

Six of the ten linkage groups, 2, 3, 5, 6, 7 and 10, were associated by this means with their respective chromosomes. Before studies with other groups were completed two new methods of associating linkage groups with particular chromosomes became available. The first method involved mutual translocations between non-homologous chromosomes. Through natural causes (13, 14, 15, 16, 19, 20, 21, 27, 57) or through X-ray treatment (2, 3, 4, 26, 28, 45, 48, 60, 62), breaks in chromosomes can be produced. If breaks occur simultaneously in each of two chromosomes, a 2-by-2 reunion of broken ends of one chromosome with those of the other chromosome may occur. In other words, a broken end of one chromosome can become attached to a broken end of the other chromosome and the remaining broken end of the first chromosome can become attached to the remaining broken end of the second chromosome. As a result of this mutual translocation, two chromosomes can be produced with new morphologies and new gene rearrangements.

In the meiotic prophase of a plant arising from fusion of a gamete possessing two normal chromosomes with one possessing these two interchanged chromosomes, the homologous associations of parts of these chromosomes produces a cross-shaped synaptic<sup>2</sup> configuration involving all four chromosomes. Thus through meiotic prophase studies, it is possible to determine not only which two chromosomes are involved but where the break occurred in each chromosome. It is obvious that such translocations of parts of chromosomes will involve transfer of blocks of genes. As a result, the linkage relationships of specific genes will be decidedly altered. The first case in which an interchange was serviceable in associating a chromosome with a linkage group involved a mutual translocation between chromosomes 1 and 2 (13, 14, 15). Genetic studies had indicated that genes of the two linkage groups *P-br*

and *B-lg* (see figure 2) were involved. Cytological studies indicated that chromosomes 1 and 2 were concerned in the interchange. Since trisomic evidence had shown that genes of the *B-lg* linkage group were associated with chromosome 2 and that genes of the *P-br* linkage group were not associated with chromosome 2, it was an obvious conclusion that genes of the *P-br* linkage group were associated with chromosome 1. Eventually, through the efforts of Burnham, Brink, Anderson, Clokey, and others interchanges involving every member of the complement were obtained. Through this method chromosomes 1, 4 and 9 were associated with *P-br*, *su-Tu* and *c-sh-wx* linkage groups respectively and a definite check on previous trisomic determinations was afforded.

The second new method of associating chromosomes and linkage groups determined the final association, that of chromosome 8 with the *j-ms*<sub>8</sub> linkage group. When pollen containing the normal allelomorph of 'japonica' was X-rayed and placed on the silks of plants homozygous for the recessive gene, 'japonica' (*j*), occasional individuals resulted which showed the recessive character. Examination of the chromosomes of one of these plants at the mid-prophase of meiosis showed that there had been a loss of a portion of chromosome 8 brought in by the male parent. This indicated that the deficiency in chromosome 8 included the dominant allelomorph of 'japonica.' The loss of the dominant gene allowed the recessive allelomorph in the normal chromosome from the female parent to express itself. This method served to establish the final association between chromosomes and linkage groups (47).

#### THE LOCATION OF GENES WITHIN THE CHROMOSOME

Two of the methods which were useful in associating linkage groups with particular chromosomes, those involving translocations and deficiencies,<sup>5</sup> were further useful in determining the physical position of a particular gene locus within the chromosome which carries it. The theory to account for crossing-over had postulated a linear arrangement of the genes along the lengths of the chromosomes. The evidence obtained from combined cytological and genetic studies in *Drosophila* had given definite proof of this organization. If genes are distributed along the lengths of a chromosome, a break in a chromosome such as occurs in mutual translocation<sup>5</sup> should sever the gene string into two blocks. Just which

genes each block would contain would depend upon where the break had occurred in the chromosome. In a mutual translocation, therefore, blocks of genes from the two chromosomes should be interchanged, the genes included in each block depending upon the position of the breaks in the two chromosomes involved in the interchange.

It is possible by studying synaptic configurations produced by the association of two normal with two interchanged chromosomes in a plant heterozygous for a mutual translocation to determine, in most cases, where the breaks occur in the two chromosomes involved in the interchange. Since the point of breakage is as useful as a gene in determining linkage relationships,<sup>14</sup> the order of known genes, within the chromosomes involved, from the point of breakage can be determined. As an example, the genes in a particular chromosome can be represented in their linear order as A B C D E F G as determined by genetic studies. Should a mutual translocation between this chromosome and another occur severing the chromosome between the genes C and D, the order of the genes with reference to the point of breakage would be in one interchanged chromosome, A-B-C-break, in the second interchanged chromosome, G-F-E-D-break. Cytological examination would reveal the position within the chromosome itself where the break had occurred but it would not indicate which block of genes was carried by each interchanged chromosome. If, however, a second translocation involving this chromosome with another were available which severed the gene string at another point, say between genes D and E, then gene D must lie between the point of breakage in the first translocation and that in the second, and the order of the genes within the chromosome with reference to the points of breakage is established. By combined cytogenetic study of many such translocations it is possible to determine with increasing precision the physical location of a gene within a chromosome. A large amount of this type of evidence has been accumulated within the past few years, some of which is published (44, 16, 45, 2, 19, 25, 28, 57, 56, 55, 59, 62, 63, 23, 24) and much of which is as yet unpublished but available to maize investigators.

A second means of determining positions of genes in the chromosomes is given by the deficiency method which was illustrated in the placing of the gene 'japonica' in chromosome 8. The piece

deleted from the chromosome contributed by the male gamete may vary from a very small to a very large section in the different cases. The extent and position of the deletion is determined by the synaptic figures produced as the result of the association of the long normal chromosome contributed by the female with the shorter (deleted) chromosome contributed by the male. With more or less accuracy depending upon the deletion involved, the positions of several genes have been located within the several chromosomes (45, 47, 59, 28, 62).

Still a third method of determining location of genes within a chromosome is available in the study of fragment chromosomes. When such fragments are present in chromosome complements in addition to the normal diploid number twenty it is possible to determine by cytological observations the region of a particular chromosome with which the fragment is homologous (46 and Rhoades, unpublished). Genetic studies will determine which genes of a linkage group are included in the fragment and which are excluded. The smaller the fragment carrying known genes, the more accurate the determination of the physical positions of the genes within the chromosome from which the fragment arose.

It can be seen that our knowledge of the association of chromosomes with linkage groups and the positions of genes within the chromosomes has developed as a result of the fitting together and correlation of information obtained by the various investigators working with several different methods.

#### CYTOLOGICAL AND GENETICAL STUDIES ON CROSSING-OVER

The placing of genes in linkage groups and locating them on the chromosomes involves a study of the process called "crossing-over." The term "crossing-over" is used to denote the exchange of pieces or segments between homologous chromosomes. There are many facts which indicate that exchange of parts or segments occurs during the first meiotic prophase when the two homologous chromosomes are in intimate association. During the early stages of meiosis the chromosome derived from the paternal parent pairs with its homologue from the maternal parent. This association of the two homologues continues until disjunction<sup>4</sup> occurs during anaphase 1.<sup>3</sup> The phenomenon of crossing-over is effected at some time between synapsis and disjunction. The exact time at which



crossing-over takes place is a controversial matter but it most probably occurs either at pachytene or diplotene. If we assume that the chromosome derived from the paternal parent carries genes  $A$  and  $B$  and that the maternal chromosome carries the recessive allelomorphs,  $a$  and  $b$ , we can illustrate the genetic phenomenon of crossing-over. If genes  $A$  and  $B$  are so situated in the chromosome that no crossing-over occurs between them there are only two gametic types possible, i.e.,  $A$  is always associated with  $B$  and  $a$  with  $b$ . If, however, crossing-over does occur so that  $a$  comes to lie in the same chromosome with  $B$  and  $A$  lies with  $b$ , two new chromosomes arise, namely,  $aB$  and  $Ab$ . These latter two types are called new combinations since the constitution of these chromosomes differs from those derived from the two parents. The gametic types  $AB$  and  $ab$  are called parental combinations since they are identical with the two chromosomes obtained from the parents. The amount of crossing-over between the linked genes is based upon the relative proportions of parental and new combinations. If ten per cent of the tested chromosomes from an  $F_1$  heterozygote<sup>15</sup> are new combinations and 90 per cent are parental combinations then the cross-over value between  $A$  and  $B$  is ten per cent and on the genetic map they would be placed ten map units apart. It should be apparent from the above illustration that the linear locations of genes in the genetic maps of figure 2 are based on the amount of crossing-over between the different linked genes.

In the earlier days it was supposed that crossing-over between two paired chromosomes occurred before they had split equationally (lengthwise). *Drosophila* workers have shown, however, that each chromosome is split into two effective parts so that there are four strands (chromatids) present when crossing-over takes place. They were also able to show (1) that in diploids, at least, only two of the four strands at any one point were involved in an exchange of genes. These detailed studies on the mechanism of crossing-over were obtained genetically as it is impossible to study the early meiotic stages in *Drosophila*. Since, from each point of crossing-over there are two cross-over chromatids and two non-cross-over chromatids it follows that the number of points at which crossing-over occurs must be twice as great as the amount of recombination observed by genetic studies. That is, if genes  $A$  and  $B$  are 10 units apart on the genetic map there was a point of crossing-over

between the two genes in twenty per cent of the sporocytes.<sup>16</sup> In *Zea*, as in *Drosophila*, it has been possible to demonstrate that chromosomes are split equationally at the time crossing-over takes place and that crossing-over occurs between chromatids; this demonstration has been made not only by genetical studies but also by direct cytological observation.

Genetic demonstration of chromatid<sup>17</sup> crossing-over rests on the following facts. When trisomic plants carrying two dominant and one recessive allelomorph are back-crossed there appear in the progeny some trisomic plants which carry the recessive gene in all three chromosomes. In order that an  $n+1$  egg could receive two chromosomes both of which carry the recessive allelomorph, crossing-over must have occurred between chromatids and not between whole chromosomes. The frequency of these exceptional trisomic plants varies for different loci. The recessive gene,  $bm_1$ , never or rarely is found homozygous in a trisome from a cross of the above type, while  $v_2$  trisomes are found with more than twice the frequency of  $pr$  trisomes. These different frequencies are intelligible if it is assumed that  $bm_1$  lies closer to the insertion region than do  $pr$  and  $v_2$  since the appearance of the exceptional trisomes is due to chromatid crossing-over between the locus of the gene in question and the insertion region. That  $bm_1$  is situated in the short arm of chromosome 5 close to the insertion region with the  $pr$  locus in the long arm but some distance away from the insertion region and the  $v_2$  locus beyond  $pr$  has been shown by cytogenetic studies with translocations, ring fragments, etc. These studies support the prediction based on the frequency of exceptional trisomes as to the relative positions of these three loci with respect to the insertion region (58 and Rhoades, unpublished).

Cytological proof of chromatid crossing-over was obtained in 4 ways but only two of them will be discussed here. Certain strains of maize have an inverted section in the short arm of chromosome 8. In plants heterozygous for the inversion<sup>18</sup> loop shaped configurations result at pachytene from the 2-by-2 alignment of the homologous loci within the inverted region. Crossing-over between two non-sister chromatids within the inversion gave at metaphase 1 one chromatid with two insertion regions, one fragment chromatid with no insertion region and two unmodified chromatids. When a second cross-over occurred in the inverted region between

the other two chromatids there resulted at metaphase I two chromatids with two insertion regions each and two fragment chromatids with no insertion region (47).

A second cytological demonstration came from study of the position of the terminal knob on chromosome 9 when this chromosome had been involved in a reciprocal translocation<sup>19</sup> which gave a ring of four chromosomes at diakinesis. One chromosome 9 had a large knob on one end and a translocated piece on the other. The other chromosome 9 had a small knob and no translocated piece. The terminal knobs of the two chromosomes were associated end to end at diakinesis but it was observed in some sporocytes that one small-knobbed chromatid had exchanged partners with one large-knobbed chromatid. That the exchange took place between chromatids and not between whole chromosomes was shown by the synaptic relations of the entire translocation complex (30).

Although crossing-over has been defined as the exchange of parts or segments between homologous chromosomes it was not until 1931 that cytological corroboration of this genetically reasoned fact was demonstrated. It is true that the great mass of genetic data in such forms as *Drosophila* and *Zea* had led geneticists to the conclusion that such a physical exchange of segments must occur, but material suitable for the demonstration of a correlation between cytological and genetical crossing-over was lacking. Creighton and McClintock (29, 31) and Creighton (unpublished), utilizing a unique cytogenetic set-up in *Zea*, were able to show such a correlation. Stern (64) made a similar demonstration in *Drosophila*. Certain strains of maize have a large terminal knob on the short arm of chromosome 9 while other strains are knobless. This knob is inherited from generation to generation in the same manner as a gene. There also exists a reciprocal translocation between chromosomes 9 and 8 in which most of the long arm of chromosome 9 has been interchanged for a segment of the long arm of chromosome 8. The genes *yg<sub>2</sub>*, *c* and *wx* lie in the region between the point of interchange in chromosome 9 and the terminal knob with *yg<sub>2</sub>* lying extremely close to the terminal knob. A plant heterozygous for the terminal knob, the genes *yg<sub>2</sub>*, *c* and *wx*, and the reciprocal translocation, was crossed with a plant with no knob, homozygous for *yg<sub>2</sub>*, *c* and *wx* and carrying unmodified chromosomes 9 and 8. The progeny from this cross were classified on the basis of genetic

cross-overs and a cytological study of these plants was made at microsporogenesis<sup>20</sup> to see if the two heteromorphic points (the knob and the point of translocation) tended to be exchanged when a genetic cross-over in closely adjacent regions occurred. They found, for example, that when a genetic cross-over between  $yg_2$  and  $c$  occurred the knob on the chromosome carrying  $yg_2$  always was transferred to the other chromosome along with  $yg_2$ . A similar correlation was found for the other regions. Recently Brink and Cooper (19), using a different cytogenetic set-up in maize, reported data which substantiates this correlation of genetical and cytological crossing-over.

That there is a close correlation between crossing-over and end-to-end association at diakinesis has been shown by Beadle (11) in his studies on *Zea-Euchlaena* hybrids. In *Zea* the paired chromosomes are synapsed<sup>1</sup> throughout their lengths at pachytene<sup>21</sup> but at diakinesis<sup>3</sup> and metaphase I<sup>8</sup> the two chromosomes open out or fall apart so that they often are associated only at their two ends. This opening out of the two homologues begins at diplotene<sup>21</sup> and is completed by diakinesis. It is known in *Zea* (47) that chromosome segments which are non-homologously paired at pachytene fall apart in diplotene and this failure to remain associated is correlated with a lack of crossing-over. If, then, association at diakinesis is found only when genetic crossing-over occurs it would be expected that no end-to-end association at diakinesis would be found between two arms of homologous chromosomes in which no crossing-over occurred. When the maize chromosome was present with its teosinte<sup>22</sup> homologue, Beadle found that no crossing-over occurred in the short arm of chromosome 9 and that the ends of the two short arms never were associated at diakinesis. Although Beadle reported in his observations at pachytene that the two short arms were usually in close association Creighton (unpublished) found in comparable material that non-conjugation or irregular conjugation was common between the two short arms. Her observations suggest that some structural difference between the *Zea* and *Euchlaena* chromosomes is responsible for the failure of crossing-over to take place.

Chiasmata are the places where exchanges of partners among the four chromatids have been observed cytologically. Genetic crossing-over may or may not occur at these places. Since, how-

ever, crossing-over has been shown to be related to post-diplotene association it would seem that chiasmata frequencies should also be related to post-diplotene association if there is any correlation between chiasmata and crossing-over. Beadle in his *Zea-Euchlaena* hybrids found that post-diplotene association was confined to a particular segment in which genetic crossing-over was known to occur and where chiasmata existed at diakinesis. Moreover, the frequency of chiasmata in this segment was roughly twice the amount of crossing-over. These data indicate some relationship between chiasmata formation and genetic crossing-over.

#### REARRANGEMENTS OF PARTS OF CHROMOSOMES

Brief mention has been made above of rearrangements of segments of chromosomes resulting from natural causes and from X-ray treatment. At this point an attempt will be made to describe the types of rearrangements which have been found and investigated. The evidence so far obtained suggests that most of these rearrangements can be explained by assuming that broken ends of chromosomes tend to unite 2-by-2. When, through natural causes or through X-ray treatment, a chromosome is broken at any one place, two broken ends are produced. If, simultaneously, the same chromosome at another position or another chromosome is broken, two more broken ends are produced. These broken ends then unite 2-by-2. The type of rearrangements obtained from such breaks and reunions will depend altogether upon which two broken ends reunite. It is altogether probable that the breaks and reunions in chromosomes occur between sections of chromosomes which are lying adjacent to one another at the time the breaks occur. The diagram in figure 3 will illustrate the categories of rearrangements expected on this assumption. In *a*, figure 3, a section of a chromosome is looped upon itself. If breaks occur in the chromosome at the position of the arrow to produce the condition shown in *b*, there could be three possible 2-by-2 unions of ends. If end 1 is united with end 4 and end 2 with end 3, no alterations would occur in the arrangement of parts of the chromosomes. If end 1 united with end 2 and end 3 with end 4, an *inversion* of a section of the chromosome would occur. If, however, end 1 united with end 3 and end 2 with end 4, two chromosomes would be formed. The union of end 1 and 3 would result in a rod-shaped chromosome with a

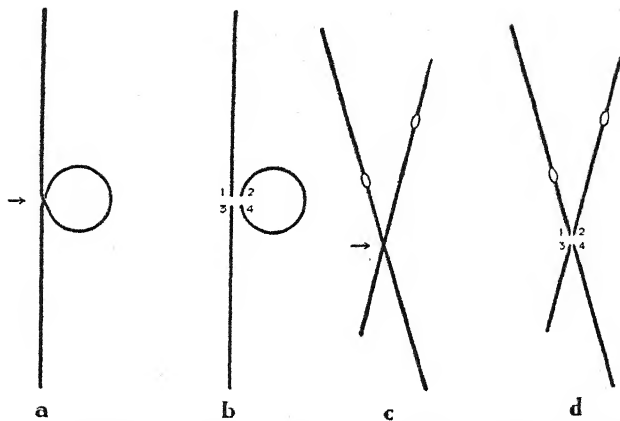


FIG. 3. Diagrammatic representation of origin of chromosomal rearrangements through the union of broken ends.

section *deleted*. The union of end 2 with end 4 would produce a *ring-shaped* chromosome, composed of a section of the normal chromosome. The evidence accumulated so far from studies of both plant and animal material has indicated that chromosomes which do not possess a spindle fiber<sup>4</sup> attachment region are incapable of functioning in the spindle figure and are, consequently, lost to future nuclear generations. Therefore, which chromosome will survive in future mitotic divisions and be visible to the investigator will depend upon which section retained the spindle fiber attachment region. If the spindle fiber attachment region is in the rod-shaped piece, the ring-shaped chromosome will be lost in the mitotic divisions and the rod-shaped chromosome will continue in future mitotic cycles as a deleted chromosome. If, however, the spindle fiber attachment region is in the ring-shaped section, the rod-shaped chromosome will be lost and the ring-shaped chromosome will survive in future mitotic cycles. Should breaks occur in two chromosomes at the position of overlapping, as at the arrow in *c*, figure 3, the four broken ends as in *d*, would be formed. In this case, also, three types of unions would be expected. Union of broken end 1 with 4 and 2 with 3 would produce no rearrangement. The union of broken end 1 with 3 and 2 with 4 would result in a *mutual translocation*, a segmental interchange, between these two chromosomes. The union of end 1 with 2 and 3 with 4 would result in a chromosome with two insertion regions and a chromo-



some with no insertion region. Examples of all of these chromosomal rearrangements have been found.

The linear organization of chromosomes makes it possible for breaks to sever specific elements of the chromosome into two parts. As a result of such breaks important information has been gained regarding specific regions of the chromosome. A break which severed the reticulate region of the satellited chromosome made it possible to prove that this element was responsible for the orderly development of the nucleolus<sup>9</sup> in the telophase.<sup>3</sup> Breaks in two cases which severed the spindle fiber attachment region of chromosome 5 revealed a feature of this structure not hitherto known (46). In the meiotic prophase, the spindle fiber attachment region appears as a relatively conspicuous, lightly staining region in the chromosome. In the two cases mentioned, breaks in chromosome 5 occurred as in *b*, figure 3, which involved the spindle fiber attachment region. A 2-by-2 reunion of the broken ends took place in such a way that a ring-shaped chromosome possessing a section of the spindle fiber attachment region and a rod-shaped chromosome possessing the remaining part of the spindle fiber attachment region were produced. Both sections of the broken spindle fiber attachment region were capable of functioning in the spindle figure and, consequently, both the rod-shaped and the ring-shaped chromosomes perpetuated themselves throughout the mitotic cycles. In the first case, the ring-shaped chromosome received only a relatively small section of the original spindle fiber attachment region whereas in the second case, the ring-shaped chromosome received a larger section of the original spindle fiber attachment region.

The implications which can be derived from the evidence produced by these two cases may be useful in explaining some hitherto puzzling cases. If sections of spindle fiber attachment regions are as equally capable of functioning as the original spindle fiber attachment region, it is possible that changes in chromosome numbers can occur with no necessary change in the genomic<sup>23</sup> complement. Just what part breakage of spindle fiber attachment regions and functioning of both sections has played in the building up of higher chromosome numbers in species and genera is not known but it is a factor which must be considered in any discussion of the evolution of chromosomes and of chromosome complements.

In describing the method by which translocations occur, empha-



sis was placed on the assumption that broken ends of chromosomes tend to unite. This is not, however, purely an assumption but finds substantial support in the mitotic behavior of ring-shaped chromosomes.

Detection of ring-shaped chromosomes has been facilitated by their striking mitotic behavior which results in the production of variegation (46 and McClintock, unpublished). In order to have the variegation appear it is necessary that the normal homologues of the ring-shaped chromosome carry recessive genes, the ring-shaped chromosome carrying the dominant allelomorphs. If the ring-shaped chromosome perpetuates itself throughout mitotic cycles in its original form, as do rod-shaped chromosomes, the plant should be totally dominant in appearance. However, ring-shaped chromosomes in maize do not perpetuate themselves unaltered through nuclear generations. Losses of sections of chromatin within the ring are continuously occurring. When the section lost includes the dominant gene, all cells arising from the cell which underwent the initial loss will lack the dominant gene in their nuclei. Therefore, expression of the recessive character carried by the normal rod-shaped chromosomes will appear in this tissue. Such behavior of ring-shaped chromosomes results in variegated plants, parts of the plant tissue showing the dominant character and other parts showing the recessive character. That the ring-shaped chromosomes actually undergo striking changes in size was readily apparent from an examination of the many microsporocytes<sup>16</sup> from a single anther whose nuclei are in the same stage of prophase development. Direct comparisons of ring size in these relatively closely related cells gave evidence of great differences in size and chromatin constitution, although blocks of related cells frequently had rings of similar size and constitution. It was not possible in these stages to obtain an idea as to how this change in size and constitution was occurring. Therefore, a study of somatic nuclear division was undertaken in an effort to determine the method and possible cause of this loss of sections of chromatin within the ring-shaped chromosome. Here the method by which the changes in size are produced became apparent. The cause of this phenomenon in ring-shaped chromosomes probably relates to the method by which a chromosome becomes split into two equal halves during mitosis. Whatever the method of splitting may be (a discussion of

which will not be considered here), the end product does not always lead to two ring-shaped chromosomes lying adjacent to each other as if they had been split into two along one plane. Instead, especially with large rings, many anaphase figures reveal that the end product of the splitting process has produced two interlocking rings or one large double-sized ring with two spindle fiber attachment regions. In this latter case, the large single ring with the two spindle fiber attachment regions is built up from both split halves of the mother ring. On the simple hypothesis that the chromosome may start its splitting process at more than one place in the chromosome and that the planes of the splits so started do not correspond, it is relatively easy to see how such figures could be obtained. In both the interlocking rings and the double-sized rings, the pull on the chromosomes produced by the passage of the spindle fiber attachment regions to opposite poles introduces a strain on the chromosome which eventually leads to breakage. In the case of the double-sized ring with the two spindle fiber attachment regions, the chromosome does not always break in the middle but may break in several places, the parts adjacent to the spindle fiber attachment region at both poles passing into the telophase nuclei, the other parts being left in the cytoplasm. Obviously, then, the chromatin content and structural arrangement of the original ring has become altered. It might be expected that this mechanism would produce rod-shaped chromosomes. On the contrary, the broken ends thus produced apparently reunite to form rings. The presence of rings of various sizes in the root-tip nuclei and the microsporocytes and the fact that no rod-shaped chromosomes have been observed to have arisen from a ring-shaped chromosome make the conclusion inescapable that broken ends of chromosomes tend to reunite.

#### FACTORS AFFECTING MEIOTIC CHROMOSOME ASSOCIATION

Knowledge of the synaptic process in maize has been gained mainly as a result of studies of the association of chromosomes in complements which are unbalanced either numerically (monosomic, trisomic) or structurally (heterozygous for an inversion, a translocation). The main conclusions from these studies (22, 47) can be summarized as follows:

1. Homology is the main force which controls the attraction and

movement of homologous parts of chromosomes toward one another.

2. Association once started between homologous parts of two chromosomes tends to continue along the chromosome bringing successive regions into intimate association.

3. When three homologous chromosomes or sections of chromosomes are present in a nucleus, homologous associations take place only between two of the three homologous elements, *i.e.*, synaptic association is 2-by-2.

4. There is a tendency for all parts of all the chromosomes to be associated 2-by-2 at the meiotic prophase period whether or not the parts associated are homologous.

In ordinary diploids, factors 1, 2 and 4 all work in harmony to produce an intimate side-by-side association of homologous parts of homologous chromosomes. Each chromosome derived from the male gamete associates homologously throughout its length with its homologue derived from the female gamete resulting at the pachytene stage of meiosis in 10 double or bivalent chromosomes. In numerically and structurally unbalanced complements, these factors which in a diploid produce homologous associations of the chromosomes come into serious conflict. The nature of the conflict may best be illustrated by describing some of the synaptic configurations obtained from these plants.

In trisomic individuals the three homologous chromosomes must compete with one another in the production of 2-by-2 homologous associations (3, above). One of the chromosomes may be homologously associated with a section of a second and at another place homologously associated with a section of the third. In all cases, a section of one of the chromosomes, or even a whole chromosome, where 2 of the three chromosomes have synapsed completely throughout their lengths, does not have homologous regions with which to synapse. The tendency for 2-by-2 associations in the meiotic prophase (4, above) of maize frequently forces the unpaired section in the first case, or the whole chromosome in the second case, into a 2-by-2 association within itself, *i.e.*, fold-backs occur bringing non-homologous parts of this chromosome into intimate association. There is no distinction as to where the folding occurs or as to which two parts become so associated.

Monosomic ( $2n - 1$ ) plants illustrate factor 4 exceptionally well.

In these cases one chromosome has no homologue with which to synapse. Although a few figures show the univalent unassociated throughout its length, most figures reveal one or more fold-backs at varying positions within the chromosome and involving from short to long sections of the chromosome.

Conflict resulting from the operation of factors 1 and 2 is strikingly illustrated in the configurations in plants heterozygous for deficiencies, translocations and inversions.

In plants heterozygous for an internal deficiency, the deficient chromosome is shorter than the normal chromosome by the extent of the deficiency. The association of the shorter chromosome with the longer chromosome necessitates the longer chromosome's buckling at some position to compensate for the deficiency within the shorter chromosome. If factor 1 operates solely in bringing parts of these two chromosomes together, the position of the buckle would be constant and would correspond exactly to the location of the deficiency within the shorter chromosome. Actually, in maize, there is a wide shifting in the position of the buckle from the theoretical expectancy based on homologous associations only. This shifting results from the operation of factor 2 working in conflict with factor 1. Two-by-two associations may start, for example, between homologous ends of these two chromosomes. Once commenced, this 2-by-2 association may travel along the chromosome, past the point of homology into the region of non-homology before meeting the 2-by-2 association travelling from the opposite direction, thus automatically shifting the position of the buckle from the region of the deletion. This results in a more or less extensive amount of intimate non-homologous association depending upon the degree of shift of the buckle from the theoretical expectancy on the basis of homologous associations only.

This same explanation also applies in interpreting variation in position of the center of the cross-shaped synaptic configuration resulting from association of the two normal chromosomes with the two interchanged chromosomes in plants heterozygous for a mutual translocation. Strictly homologous associations in such cases, *i.e.*, operation of factor 1, would produce cross-shaped configurations the centers of which would mark the position where the breaks had occurred in each of the two interchanged chromosomes. The operation of factor 2, however, results in some cases in a con-

siderable shift in the position of the center of the cross. Any shift away from the theoretically expected center involves the association of non-homologous parts of chromosomes.

The conflict between factors 1 and 2 is rather interestingly illustrated in the synaptic configurations obtained from plants heterozygous for inversions. In order to bring about associations of homologous parts of the normal chromosome with those of the inverted chromosome, the chromosomes must undergo considerable movement within the nucleus. Factor 1, *i.e.*, the force of homology, controls this movement. If the inversion represents a relatively large section, the combined forces of homology within this region are sufficiently strong to insure a movement of the chromosome into a loop and the bringing together of homologous parts of these two chromosomes within the region of the inversion. As the relative size of the inversion becomes smaller, the combined effective forces of factor 1 in the region of the inversion likewise becomes smaller. In these cases, associations first initiated between regions outside of the inverted section tend to continue along the chromosome and even prevent loop formation within the region of the inversion. Thus the sporocytes of plants heterozygous for short inverted sections of a chromosome frequently show no synaptic evidence of the inversion, the two chromosomes being associated in the form of a rod. In these cases, the association in the region of the inversion is strictly non-homologous.

The consequences of such non-homologous associations as described above are several. Cytological studies have shown that sections of chromosomes which are associated non-homologously at pachytene usually separate from one another completely early in the diplotene stage without any obvious consequences arising from the former association. One would expect, therefore, that cross-over values in specific regions which are sometimes undergoing homologous and sometimes non-homologous associations in different sporocytes of the same plant would show marked reduction in cross-over values for the genes located in these regions. Burnham (23) and Stadler (63) have reported cases of reduction in crossing-over which can be directly related to non-homologous associations.

Although non-homologously associated parts of chromosomes usually are seen to separate completely from one another during

the diplotene stage, the progeny from individuals in which non-homologous associations of a certain type are known to have occurred, give evidence that occasionally translocations occur between these parts which are non-homologously associated. In other words, new types of modified chromosomes occur in the progeny whose origin can be explained by translocations which have taken place between regions of chromosomes known to be non-homologously associated in the parent plant. In the progeny of trisomics, for example, rod-shaped chromosomes with deletions or ring-shaped chromosomes representing a section of one of the three homologous chromosomes of the trisomic, have appeared (Rhoades, unpublished; McClintock, unpublished). If one considers that the deleted rod-shaped chromosome and the deficiency ring-shaped chromosome arise from translocations occurring in fold-back univalents (see page 309) they are readily seen to be reciprocals of one another, the position of the spindle fiber region with reference to the points of translocation determining which chromosome, the ring or the rod, will survive. More direct evidence that translocations occur in regions non-homologously associated has recently been presented by Stadler (63). As stated above (page 310) the synaptic association of a normal chromosome with one possessing an internal deficiency produces a buckling in the normal chromosome to compensate for the loss of a segment in the deficient chromosome. The position of the buckle can vary over a considerable range of the chromosome. Since only one position of the buckle represents complete homologous associations, any shift from this position involves non-homologous associations. Since there are many such cases, there is ample opportunity for non-homologous parts to be associated. A translocation (or cross-over) occurring in this region would result in a short chromosome with a shifted deficient region and a chromosome of normal length but with a deleted and a duplicated section. By appropriate genetic means, Stadler has been able to detect such modified chromosomes.

#### ANEUPLOIDY AND EUPLOIDY

The normal somatic complement in maize, as has been stated above, is twenty chromosomes. There are, however, various modifications of this normal complement. Entire haploid sets of chromosomes may be added or subtracted to give a euploid series. The



addition to the normal complement of one or more chromosomes, which may be either unchanged or modified in various ways, gives hyperploid types. Hypoploids are those strains in which a chromosome or part of a chromosome is missing. A plant may also be hyperploid for a section of one chromosome and hypoploid for a section of another.

The simpler types of hyperploids will be considered first. Strains of maize which have nine of the ten chromosomes in duplicate and the tenth in triplicate are called primary trisomes since the supernumerary chromosome is identical with its two homologues. Only eight of the ten possible primary trisomes in *Zea* have been isolated at the present time. The missing primary trisomes are for chromosomes 1 and 4. It has been possible to associate specific differences in the appearance of trisomic plants with the presence of certain chromosomes which are in triplicate. This is true of plants trisomic for chromosomes 2, 3, 5, 7, and 8. The primary trisomes in *Zea* are all, however, characterized by the fact that they are smaller and less vigorous than their disomic sibs.<sup>24</sup> The effect of an extra chromosome on the appearance of a plant was first shown by the work of Blakeslee *et al* on primary trisomes in *Datura* where the unbalance in the genic complement produced by the addition of an extra chromosome resulted in a changed appearance of the plant. There are twelve primary trisomes in *Datura* and as each of the twelve chromosomes contains a different packet of genes it might be expected that the primary trisomes would differ phenotypically<sup>25</sup> from one another. Such indeed is the case and the *Datura* workers can recognize the different primary trisomes by their characteristic appearances.

A secondary trisome differs from a primary in that the extra chromosome is not a replicate of one of the members of the monoploid set but has become modified so that its two arms are identical. Chromosome 5, for example, has an insertion region which is nearly median. We can represent the shorter arm by  $\alpha$  and the longer arm by  $\beta$ . There are two secondary trisomes possible for chromosome 5. In one case the supernumerary chromosome is composed of two  $\alpha$  arms, and in the other case it may be composed of two  $\beta$  arms. Only one secondary trisome has been found in maize. Cytological studies at pachytene in meiosis show that it was the  $\alpha$ - $\alpha$  secondary (58). This secondary trisome differed



markedly in appearance from its disomic sibs. It had in an accentuated degree certain of the characteristics which distinguish the primary trisome of chromosome 5. This accentuation of certain of the primary trisome characteristics is due to the fact that in the secondary the short arm of chromosome 5 is present in quadruplicate and the long arm in duplicate while in the primary trisome the short and long arms are both in triplicate. This piling up of genes of the short arm produces a different genic unbalance which is reflected by an exaggeration in the secondary of certain of the primary trisomic characters which presumably are conditioned by the genes in the short arm of chromosome 5.

A tertiary trisome is one in which the extra chromosome is composed of parts of two different members of the monoploid set. The numerous tertiary trisomes in maize have been derived from plants heterozygous for a reciprocal translocation in which a 3 to 1 distribution of the four members of the ring at anaphase 1 results in a functional eleven chromosome gamete.

These three types of  $2n+1$  plants give, in addition to trisomic ratios for those genes which are included in the reduplicated sections, a range of synaptic configurations at diakinesis which is in accord with the theory that only homologous ends of chromosomes are associated at this stage.

*Deficiencies.* In another section of this paper the production of rod-shaped chromosomes with an internal deletion and ring-shaped chromosome fragments through translocation in univalent chromosomes has been discussed. Most of the deficiencies in *Zea* have been produced through the agency of X-rays.

Most of these X-ray induced deficiencies are incapable of being transmitted through either the eggs or pollen but a few of them are inherited through the eggs. These are called haplo-viable deficiencies. Stadler (62) who has obtained several such deficiencies reported one for chromosome 10 in which approximately one-third of the long arm including the locus of *R* was missing. Pollen from plants heterozygous for this deficiency was of two sizes. Those grains with the deficient chromosome were smaller than normal, only partially filled with starch and were incapable of sending forth a germ tube when placed on fresh silks while the other class of pollen containing the full genomic complement was normal in size, appearance and behavior. Female gametophytes with the

deficient chromosome were also smaller. However, some of these gametophytes developed embryos as the result of fusion of the deficient nuclei of the female gametophyte with the nuclei of male gametophytes containing complete haploid sets of genes. Plants heterozygous for the deficient chromosome were also of reduced stature, presumably because of the chromosomal unbalance produced.

Failure of chromosomes with a deficiency to be transmitted through pollen when they may be carried through eggs is due to lack of competition between female gametophytes while deficient pollen must compete in a race down the long maize styles with normal pollen. Deficient pollen is often incapable of germinating. If it does, the rate of pollen tube growth might be slowed down sufficiently to mitigate any chance of achieving fertilization. It should be noted that in the case of deficiencies it is lack of chromatin essential for normal development which prevents functioning of the male gametophyte while in trisomic types it is the presence of duplicated chromatin which is responsible for failure of  $n+1$  pollen to function. The underlying cause is the same in both cases, *i.e.*, the unbalance produced either by a deficiency or a duplication is too great a handicap for successful competition with normal pollen. It is entirely possible that a small deficiency, including no essential genes, could be transmitted through both pollen and eggs.

Burnham (21) found in his studies with a reciprocal translocation involving chromosome 1 and chromosome 6 that eggs having duplication for a considerable portion of chromosome 1 and deficient for the terminal chromomere<sup>26</sup> of the satellite of chromosome 6 were viable. Pollen of the same constitution was non-functional but was well filled with starch.

In the above discussion of supernumerary chromosomes only those types have been discussed in which the extra chromosome represents some modification of one or more members of the normal monoploid set of ten. There has been found, however, in certain strains of maize, especially Black Mexican sweet corn, a type of supernumerary chromosome which is totally unlike any members of the regular complement. It cannot be said, on the basis of its peculiar morphology, to have been derived from any one member of the monoploid set and its origin is unknown. This type of

supernumerary has been called the *B*-type chromosome in contradistinction to members of the normal complement which Randolph (50) has designated as the *A*-type chromosome. The *B*-type has a distinctive morphology quite unlike any of the *A*-type chromosomes. It appears to be composed of genetically inert material and carries no known genes. The presence of one or many *B*-type chromosomes has no visible effect on the morphological character of the plant. Randolph has succeeded through successive crosses in accumulating more than twenty-five *B*-type chromosomes in a single plant in addition to the regular complement of twenty. In contrast with supernumeraries composed of *A*-type chromosomes the *B*-type is readily transmitted through both pollen and eggs.

The morphology of the *B*-type chromosome at pachytene and its synaptic behavior have been investigated by McClintock (47). In the meiotic prophase the *B*-type is slightly more than one-half the length of the shortest member of the normal complement. Its morphology at mid-prophase, beginning with the terminal insertion region, is as follows: (1) terminal spindle fiber attachment region, (2) pycnotic<sup>27</sup> region, (3) chromatic region composed of small but distinct chromomeres, (4) elongate pycnotic region with several definite constrictions, (5) bulging pycnotic region, (6) broken pycnotic region composed of four distinct parts. The greater part of the *B*-type at mid-prophase is composed of pycnotic material. As stated before, there is reason to believe that the *B*-type chromosome is genetically inert. That these two facts have some close relationship is suggested by Heitz's studies (37, 38) with *Drosophila* in which he shows that the pycnotic portions found in prophase chromosomes are genetically inert.

The *B*-type shows no synaptic affinity for any of the chromosomes composing the normal set. If a single *B*-type is present it behaves as a univalent but regions within it are often non-homologously paired at mid-prophase in meiosis. Synapsis occurs between *B*-type chromosomes if two or more are present in the same nucleus although non-homologous association is very common. McClintock found that in plants with two *B*-types there were more sporocytes with two univalent *B*-type chromosomes at diakinesis than there were in mid-prophase where the *B*-types were usually paired. This has been attributed to the complete separation of the two members of a *B*-type bivalent during diplotene and early diakinesis

which occurs in some sporocytes. This precocious separation may be due to frequent occurrence of non-homologous association observed at pachytene and/or to partial failure of chiasma formation when homologous pairing does occur.

In the euploid series in maize the number of complete haploid sets of chromosomes ranges from one to eight. A haploid has one, a diploid two, a triploid three, a tetraploid four, and an octoploid eight complete sets of the ten chromosomes. Haploid and triploid maize plants occur spontaneously while tetraploids and octoploids have been produced (Randolph, 51) by the doubling and quadrupling of the chromosome number through the application of heat to young ears at the time of the first divisions of the zygote. Triploids can be obtained by crossing tetraploid with diploid maize but this cross is highly sterile and few seeds result. In contrast with hypo- and hyperploid maize where visible difference in the morphology of the plant often results from the chromosomal unbalance there is no striking external morphological difference between different euploid types except that the haploids are smaller and weaker plants. This is intelligible since the genic balance between the different chromosomes has not been altered as all loci are present in the same relative proportions throughout the euploid series. There is, however, a striking correlation between cell size and the number of times the haploid complement is replicated in the nucleus. Beginning with haploids, which have the smallest cells, there is a graded series ending with octoploids, which have the largest cells.

There has been, as yet, little genetic work with tetraploid maize as Randolph (52) found that, due to irregularities in chromosome distribution in meiosis, the offspring of tetraploids do not always have forty chromosomes.

*Hybrids of Zea with Euchlaena and Tripsacum.* There are two closely related genera, *Euchlaena* and *Tripsacum*, which have been successfully crossed with *Zea*. There are three annual strains of *Euchlaena mexicana*, each with a haploid complement of ten chromosomes. These three annual strains, referred to as the Chalco, Durango and Florida forms, cross readily with maize and the  $F_1$  plants are fertile. The homology between the *Zea* and *Euchlaena* chromosomes must be very close since studies of these hybrids made by Emerson and Beadle (8, 35) show that the amount of

crossing-over is essentially of the same order in all tested regions as in pure maize with the notable exception of the short arm of chromosome 9 in the Durango and Florida hybrids where no crossing-over occurred.

*Tripsacum* crosses much less readily with *Zea* than does *Euchlaena*. Both the diploid form ( $2n=36$ ) and the tetraploid form ( $2n=72$ ) of *Tripsacum dactyloides* have been successfully crossed with *Zea* by Mangelsdorf and Reeves (39, 40). The  $F_1$  hybrids of *Zea* ( $n=10$ ) by the diploid *Tripsacum* ( $n=18$ ) had twenty-eight chromosomes while the hybrids of *Zea* with tetraploid *Tripsacum* ( $n=36$ ) have forty-six chromosomes. In the first case the chromosomes show no synaptic affinity and are present as 28 unpaired chromosomes in meiosis but in the second type the 36 *Tripsacum* chromosomes form 18 bivalents with 10 *Zea* chromosomes left as univalents. The  $F_1$  of the diploid *Tripsacum* with *Zea* has a low degree of fertility on the female side but is completely male sterile. The  $F_1$  of tetraploid *Tripsacum* is both female and male sterile. The hybrid, *Zea* by diploid *Tripsacum*, has been crossed with *Euchlaena* and a trigeneric hybrid containing chromosomes from *Zea*, *Tripsacum* and *Euchlaena* was obtained.

#### SUMMARY

The combined efforts of a group of people working in close unison and interchanging results and material previous to publication has contributed greatly to the rapid advance of maize in the field of genetics and cytology. The long generation cycle of maize coupled with the usual delays in publication would otherwise considerably delay progress with this plant. As a conclusion, we wish to summarize in a numerical manner some of the outstanding contributions that this cooperative study has produced.

##### A. *The relation of genes to chromosomes:*

1. The discovery of approximately 400 genes relating to a variety of life activities ranging from gross morphological changes to those affecting cell sap color, cell wall texture and color, chemical nature of starch, chlorophyll development, disease resistance, mitotic chromosomal behavior, meiotic chromosomal behavior, cytokinesis, pollen tube growth rates, aleurone color, etc.

2. The placement of many of these genes into 10 linkage groups corresponding to the 10 chromosomes comprising the haploid complement.
  3. The association of each linkage group with a particular morphologically identifiable member of the chromosome complement.
  4. The placement of specific genes at definite positions within the physical chromosome.
- B. *Cytological proofs of genetic postulates:*
1. Cytological proof of genetic crossing-over.
  2. Cytological and genetical proof of chromatid crossing-over.
  3. Cytological demonstration of the physical location within the chromosomes of reciprocal translocations, inversions and deletions.
- C. *Cytological discoveries with genetic consequences:*
1. The analysis of factors governing the meiotic association of chromosomes.
  2. The discovery that non-homologous parts of chromosomes can be intimately associated at the meiotic prophase period.
    - a. The reduction in normal crossing-over resulting from this association.
    - b. The production of occasional translocations resulting from this non-homologous association.
  3. Diakinesis associations of chromosomes are, for the most part, strictly homologous.
  4. Analysis of the importance of different sections of the chromosomes to viability of the gametophytes by means of haplo-viable and haplo-inviable deficiencies.
  5. The unique mitotic behavior of ring-shaped chromosomes resulting in genetic variegation.
  6. The discovery that both parts of a broken spindle fiber attachment region are capable of functioning in the spindle figure.
  7. The correlation of pycnotic chromatin with genetic inertness.
- D. *Polyploidy and generic hybrids:*
1. The artificial production of polyploidy by heat treatment.
  2. The cytological and genetical analysis of *Zea-Euchlaena* hybrids.
  3. The cytological analysis of *Zea-Tripsacum* hybrids.



## EXPLANATORY NOTES BY THE EDITORS

1. For a description of these characters see 36. The bulky corn plant as ordinarily observed is the *sporophyte*; each of its cells contains two sets of 10 chromosomes each (constituting  $2n$  or diploid tissue); each of these sets represents the descendants of a single set ( $1n$ , *haploid* or *monoploid* tissue) inherited from each parent. The haploid tissues contributing these single sets at fertilization are the male gametophyte (pollen tube) and the female gametophyte (embryo sac). These gametophytic tissues are produced by and constitute minute parts of each parent and their haploid nature arises through a reduction in the number of chromosomes they receive from the diploid parent.

*Endosperm* tissue has three sets of chromosomes in each cell ( $3n$  or *triploid* tissue). This condition arises by fusion of a nucleus from the pollen tube with two nuclei from the female gametophyte. The endosperm surrounds and nourishes the developing embryo; in cereals its outer layer constitutes the *aleurone* layer which contains protein material. Certain abnormal plants, arising in a variety of ways, may have three sets of chromosomes in every body cell and are then known as *triploids*.

2. *Synapsis* is the normal pairing of chromosomes preceding their distribution to daughter cells. *Asynapsis* is the failure of this pairing; they then appear as single chromosomes, *univalents*, rather than as *bivalents*. *Synapsis* and associated cytological phenomena appear during a type of cell-division known as *meiosis* during which a reduction occurs from diploid  $2n$  to haploid  $1n$  tissue. Initial stages of all nuclear divisions constitute the *prophase* and the last stage of a *meiotic prophase* is known as *diakinesis* which immediately precedes disappearance of the nuclear membrane. An intermediate stage of all nuclear divisions is the *metaphase*.

3. For every character of a sexually produced organism a *gene* or factor is ordinarily contributed by each parent. If each gene of such a pair is the same as the other, as red and red, the progeny is then *homozygous* for that character. If the two inherited factors are not alike, as red and white, the progeny is *heterozygous*. If only one of these two unlike factors is visibly expressed to the complete suppression of the other it is said to be *dominant* and the suppressed one is *recessive*. Each parent contributes one member of each pair of chromosomes in the progeny; the two chromosomes of each such pair are *homologous chromosomes*.

4. At metaphase of a meiotic (reduction) division, homologous chromosomes are ordinarily paired and then separate, each member going into a different daughter cell. This movement constitutes the *anaphase*. At *telo-phase* the daughter nuclei become organized into new cells. *Non-disjunction* is the failure of this separation and then both chromosomes move together. *Disjunction* or separation of the paired chromosomes is directed by the *spindle fibers*.

5. *Translocations* are changes in position of a segment of a chromosome to another part of the same or of a different chromosome (Darlington). *Mutual* or *reciprocal translocations* are interchanges of segments between



different chromosomes. *Deficiencies* are losses of a segment of a chromosome.

6. See page 314 for discussion of deficiencies.

7. *Cytokinesis* is the division of the extra-nuclear portion of the protoplast. It usually takes place immediately after nuclear division.

8. See page 312 for discussion of ploidy.

9. The *nucleolus* is a body in the nucleus which disappears during nuclear division. Its substance is most probably incorporated into the matrix of the chromosomes.

10. See page 299 for discussion of crossing-over.

11. This indicates that every body cell has the normal diploid ( $2n$ ) number of chromosomes plus one extra chromosome.

12. See page 313 for discussion of trisomes.

13. This means that when corn plants with one extra chromosome ( $2n+1$ ;  $F_1$  because they were the first generation plants secured by crossing a triploid with a diploid) were self-pollinated, a certain character in the progeny appeared three times as often as its allelomorph. If, on the other hand, the plants were not self-pollinated but were back-crossed with other plants which showed the recessive character, then half of the progeny exhibited this recessive character and the other half the dominant one. A discussion of the types of ratios expected from trisomic inheritance would occupy more space than is considered pertinent to this review. Details can be obtained from 49, 56.

14. Genes, the units of heredity regarded as determinants of most characters, are borne on the chromosomes. Those on any one chromosome are said to be linked.

15. A *heterozygote* is the product of a fertilization which for one or more characters possesses opposing factors.

16. *Sporocyte* = spore mother-cell. *Microsporocyte* = pollen mother-cell.

17. A *chromatid* is one half of a longitudinally split chromosome which later becomes a daughter chromosome.

18. See page 311 for discussion of inversions.

19. See page 296 for discussion of translocations.

20. The production of microspores (pollen grains).

21. *Pachytene* is the double thread (and the stage at which it occurs) produced by pairing of chromosomes in the prophase of meiosis. It is followed by *diplotene* (Darlington).

22. *Teosinte* is the common name of *Euchlaena mexicana*, a Mexican grass regarded as the nearest living relative of maize which is unknown in the wild state.

23. A *genome* is an entire chromosome set inherited as a unit from one parent.

24. *Sibs* are sister plants.

25. *Phenotype* refers to the external appearance produced by the reaction of an organism of a given genotype with a given environment. *Genotype* is the kind or type of hereditary properties of an organism (Darlington).

26. *Chromomeres* are the smallest particles in the chromosome thread.  
27. *Pycnosis* is chromatic matter of the nucleus contracted into a deeply staining homogeneous mass (Sharp).

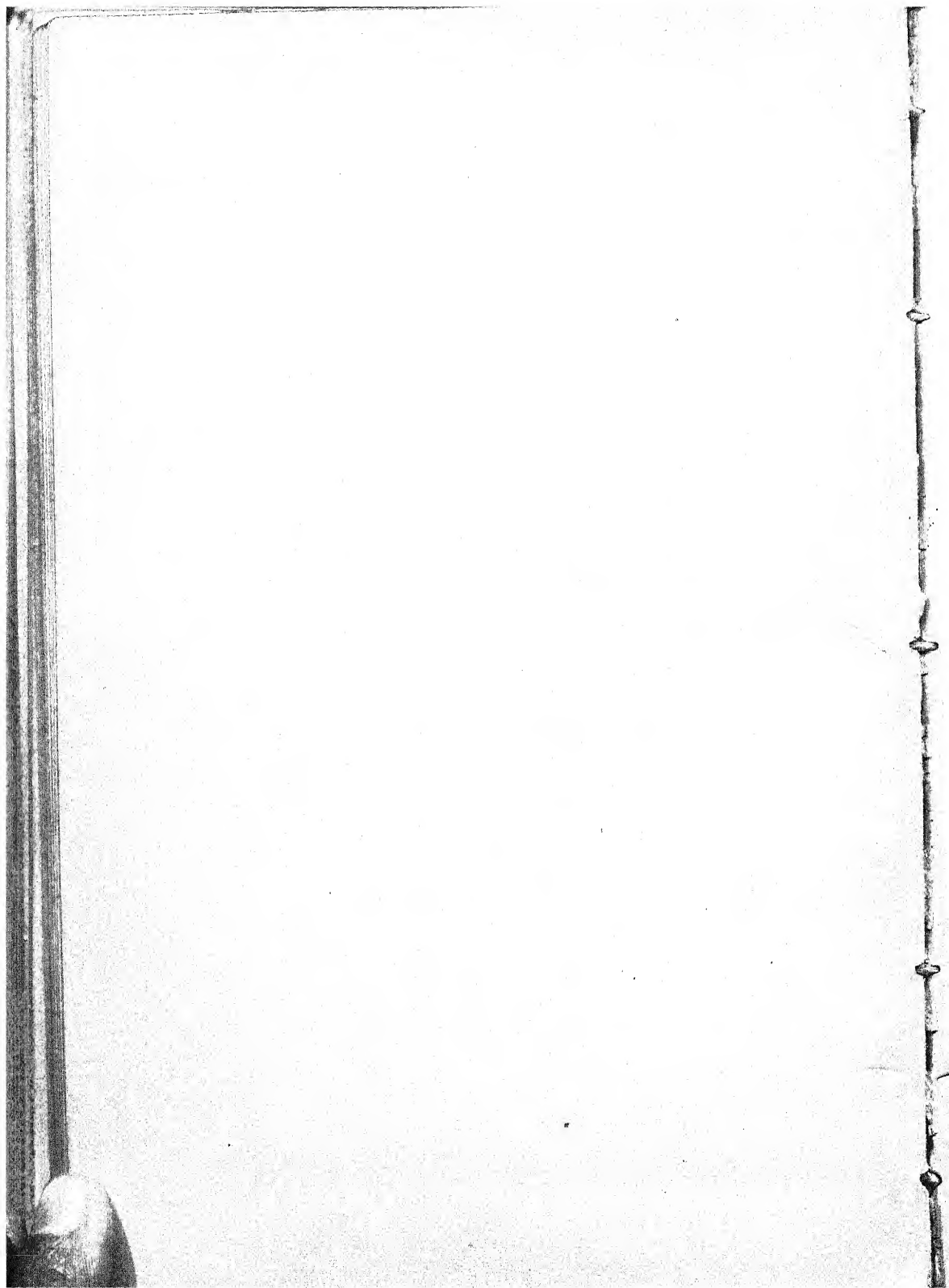
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# THE BOTANICAL REVIEW

VOL. I

SEPTEMBER, 1935

No. 9

## THE CYTOLOGY OF HOST-PARASITE RELATIONS

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I plan to discuss this topic with special reference to rust fungi. Their host relations, however, can best be appreciated against a background of those of other types of fungous parasites.

The literature covers both mycological and pathological points of view concerning fungous parasites and it is no easy problem to organize this accumulating material with respect to the physiology of parasitism. The latter is by no means a new viewpoint. It was called to the attention of mycologists by de Bary (13) who distinguished: (1) pure saprophytes, illustrated by the type of *Pilobolus anomalus*; (2) facultative parasites, illustrated by *Sclerotinia sclerotiorum*; (3) facultative saprophytes, and (4) obligate parasites, of which the Ustilagineae and the Uredineae are respective examples. In his discussion of these divisions de Bary's emphasis is upon nutrition; he sees a direct causal connection between these grades of parasitism and the process and method of feeding on the part of the fungus.

Bacteriologists, on the other hand, have emphasized invasive power. Bail (11) derives parasites from saprophytes which can under no condition colonize within foreign organisms. The tetanus bacillus is a "necroparasite" with such limited infective power that it can colonize in living organisms only through dead tissue. The infective power of "half parasites" such as the typhus organism can be easily influenced. "Pure parasites," with such high infective power that from a minimum number at entrance the spread through an organism is rapid, are illustrated by the bacilli of hemorrhagic septicemia. Theobald Smith (62) conceives a series of bacterial parasites where offensive devices of invasion give place to such a balance with one host as to allow the parasite to complete a full life cycle of invasion, multiplication and dissemi-



nation. The diphtheria bacillus is chiefly offensive; the tubercle bacillus has developed a preponderance of defensive mechanisms; the spirochaete of syphilis has dispensed largely with both offensive and defensive powers and its metabolism produces little reaction on the part of the host. Raines (56) has indicated how these functions of offence and nutrition may interact. With emphasis, as it were, upon Smith's "one host," he writes: "In the group of the fungi the transition from violent and destructive parasitism to parasitism of the symbiotic type is accompanied by a transition from facultative to obligate parasitism as if the physiological corollary of parasitism of the latter type is extreme specialization in food preferences." He illustrates the violent and destructive parasites by *Botrytis* while the seed fungus of *Lolium temulentum* he calls a benign infestation. Thus both specialization of offensive metabolism and adaptation to the living host may fall into place in de Bary's four-part classification.

There are many interesting examples of facultative parasites in the literature. *Bacterium campestre* causes the black rot of cabbage. Meier's (45) description of the effects of its intercellular masses—invagination of cell walls, disappearance of nucleoli, collapse of nuclei and chloroplastids against the cell walls and decrease in the amount of cytoplasm of the cells—proves it a successful instrument of destruction. In contrast to this type, instances of adaptation of bacterial parasites to their hosts are not lacking. Witness the classic figures of Peirce (54) showing intimate intracellular relations for the tubercle bacterium in the root cells of bur clover.

Among hyphal fungi, Pearson (53) traces the shift from saprophyte to parasite in the corn-seedling blight, *Gibberella Saubinetii*. This fungus exists as a saprophyte on crop refuse and enters the living plant only through wounds or weakened tissue. Here it is first intercellular but it may grow through the middle lamella and later invade the cells. The result is a swelling of cell walls and an accumulation of dark-staining matter in the walls resulting in dark brown, sunken lesions on the seedlings, or of rot of the entire seedling. Emmons (26) has made clear by his studies upon *Cicinnobolus Cesatii* de Bary, parasitic in *Erysiphe Cichoracearum* on *Helianthus tuberosus*, that the offensive parasite by its very success may revert to saprophytism. *Cicinnobolus* is primarily a para-

site. The invading hypha passes through the wall of the host and directly into the host protoplasm, apparently without invagination. It quickly kills the host cells, apparently by "enzymatic action rather than by toxins," if this is a real distinction. *Cicinnobolus* then continues to live upon the dead organic material. Emmons concludes: "It is specialized in its choice of host, in its method of invasion, and in the completeness of its utilization of the materials in its host." Interesting variations of the parasitism of a fungus upon a fungus are to be found in the cases of *Piptocephalis*, *Syncephalis* and *Chaetocladium*, parasitic upon other Mucorini. It was to these that Van Tieghem's (67) term "facultative parasites" was first applied but de Bary (13) considers them facultative saprophytes on the ground that "they only attain to their full development in the formation of zygospores when they live as parasites on other Mucorini."

A graded series of offensive invasions, tending finally toward mutualism, has been traced by Walker (68). *Colletotrichum circinans*, he tells us, can establish itself on onion plants only as a saprophyte on dry, outer skins of the bulb. On the dormant bulb it may succeed in an attack upon the succulent scales but only to become a mildly aggressive parasite. Toxins in the host cell, particularly in cells of the red onion, seem to hold the process in check. Possibly because of these *Colletotrichum circinans* is seldom observed as a wound parasite; it succeeds in an infection only because, after penetration of the cuticle, it causes some degeneration of the host protoplasts in advance of further penetration and thus destroys the host toxins. Walker has recently (69) isolated two water-soluble toxic substances from the outer scales of colored onions and thinks they are responsible, in part at least, for the resistance of these onions. Walker describes a more advanced type of parasitism in the case of *Botrytis byssoides*. This fungus enters usually through old tissues at the top of a bulb. Although it, too, is often inhibited by action of host cell "toxins" it does establish itself to the point where its secretions may break down host tissue in advance. Thereafter it develops rapidly upon by-products of host cell decomposition. Its secretions are thus much more potent for destruction than those of *Colletotrichum circinans*. Walker carries his series one step further into the field of adaptive parasitism by means of *Colletotrichum lindemutheanum* described

by Leach (41). In a resistant host, penetration by the fungus brings prompt disintegration of the host protoplasts and the hyphae are incapable of further development; in a susceptible host, in early stages, there is no evidence of detrimental effects upon the host protoplasts. Passage through walls is by a small opening without effect upon the adjacent wall and without effect upon the protoplast within. Only later, but quite suddenly, does there come a shift in the balance when the advancing parasite causes softening of cell walls and disintegration of cell contents. Walker concludes: "Thus in this early stage the parasite appears to maintain a relation suggestive of the higher type so common with the obligate parasites."

Of much the same nature as this series given by Walker is the series which I have traced (58) for varieties of *Botrytis*, an evolution from saprophytism to parasitism with the conclusion that "both in the occasional finer adjustment between invader and host which brings death less quickly as the result of infection, and in tendencies toward specificity in hosts, this example of a facultative parasite suggests something of the manner of life of the haustorial fungus." However, it is only facultative parasitism at best; and *Botrytis* in its host relations in general illustrates what the obligate parasite with haustoria does not do although showing a very high degree of specificity in its choice of hosts.

The morphological expression of the "extreme specialization in food preferences" which Raines has called the physiological corollary of obligate parasitism is, I believe, the haustorium. This intracellular organ of intercellular fungi exhibits great refinements in its method of wall penetration and of feeding. A compilation (58) of reports from the literature shows that all the great groups of hyphal fungi, Oömycetes, Zygomycetes, Ascomycetes and Basidiomycetes, contain species which produce haustoria. In predominantly saprophytic Zygomycetes, haustoria are reported only for one small division; in the other three groups there are subdivisions in each of which their occurrence is practically universal. Mention from these three groups of the Peronosporaceae, the Erysiphaceae and the Uredinales, respectively, as examples of haustorial groups of highly adapted parasites, is evidence that the existence of haustoria may be considered an index of the grade of specialization in the relation of host and parasite.

The feeding function of the haustorium is indicated in such quotations as the following. Latham (40) says of a *Cercospora* leaf-spot fungus of *Vigna sinensis*: "The fungus absorbs at least a part of its nutriment from the surrounding cells by means of haustoria." Weston (71) describes for *Sclerospora graminicola* on Everglade millet, in addition to slender hyphae of transmission: "knotted, much-branched, contorted, feeding hyphae which are crowded into the interstices between cells of the mesophyll and furnished with short, knob-shaped or finger-shaped haustoria." Pady (51) writes of *Hyalopsora aspidiotus*: "the haustorium is essentially a nutritive organ, making possible the growth of the organism."

With the general acceptance of the haustorium as a feeding organ, elaboration of form in the haustorium is, I believe (58), to be regarded as another evidence of a high degree of adaptation by the haustorial parasite in securing maximum contact relations with the host protoplasm. From the small button-like type of *Cystopus candidus*, the species in which haustoria were first described by de Bary (12), the literature reports endless variation of elongation, branching and coiling, culminating in such climax achievements of increased surface as the digitately-branched structures of *Erysiphe graminis* described by Smith (60) or the coral-loid structures in the Asterineae so frequently figured by Arnaud (7). A recapitulation of this development may often be seen within the life time of one species; young haustoria of *Puccinia Sorghi* are slender-stalked buttons but lobing commences early and the mature form is a digitately-lobed structure (58). It follows naturally that such highly specialized structures should have significance as specific characters. Faull (28) states with reference to the haustoria of the Pucciniastreae: "It is worth recording that they are more or less characteristic for each genus." Graff (30) uses the haustorium as the criterion by which to distinguish three grades of parasitism in the genus *Meliola*. In *Meliola circinans* Earle there is no evidence of the presence of haustoria but the superficial mycelium in some way corrodes the epidermal cell walls and causes some injury to the host; in most species, haustoria of a simple type penetrate the epidermal cells; there are also occasional species with haustoria which penetrate to the mesophyll

region. Such forms should be further studied as to their relative effectiveness in their host relations.

There are many instances from the various groups of haustorial fungi where adjustment between haustorium and host protoplast is far from effecting mutualism. Such instances may serve to show steps in adaptation to the living host. Aronescu (8), noting in *Diplocarpon Rosae* the browning of cells penetrated by haustoria, the limited surface of the haustoria and their occurrence chiefly in epidermal cells of rose leaves, says that *Diplocarpon Rosae* seems to be a transition form which has left the lowest step of parasitism but is still far from the level of a strictly obligate parasite. A *Cercospora* upon *Vigna sinensis*, according to Latham (40), causes irregular necrotic areas upon the leaves. The fungus develops rather slowly at first as an intercellular mycelium with haustoria; later, when the attacked cells have reached the necrotic condition, both inter- and intracellular mycelium may be found in the same lesion. Wolf (73) reports that *Peronospora hyoscyami* de Bary develops an intercellular mycelium in leaves of tobacco seedlings from which branched haustoria penetrate the cell walls. After spore production, not only do the invaded cells die but the collapse may involve the entire leaf or the entire plant. As a result of culture experiments, Wolf concludes that toxic water-soluble substances are formed within the diseased tissues and that they may permeate the entire plant.

On the other hand, among that group of facultative saprophytes, the Ustilagineae, where intracellular hyphae abound and haustoria apparently are not a constant feature, there are, nevertheless, striking instances of close adjustment between host and parasite. Butler's observations (17) upon the *Ustilagos* indicate much variability in the matter of haustoria. Butler reports for *Ustilago Tritici*: "growth is exclusively intercellular; there are no haustoria; and the host cells are not affected in the slightest degree by the presence of the parasite." For *Ustilago Avenae* he reports: "Except at the moment of entry, the hyphae never penetrate into the cell cavities, though they may send in haustoria." For *Ustilago Zeae* he describes "infection hyphae," either inter- or intracellular, and "feeding hyphae" which form little clumps with many branches within individual cells, comparable to haustoria of parasites that live mainly between the cells. Kolk (38) does not men-

tion haustoria for *Ustilago Avenae* in *Avena sativa* var. *Victor* (S. N. 126). She describes both inter- and intracellular hyphae and reports: "compatibility between host and pathogen reaches such a high degree of development that the fungus causes no change in the appearance of the cells, . . . and there is little difference between cells invaded by hyphae and those not." She notes, indeed, a possible stimulation of the host cell to produce more cytoplasm as the hyphae make their way through the host cytoplasm and are surrounded by it to a greater or less extent. Lutman (42) uses the rare occurrence of haustoria in *Ustilago* as a generic character over against that of well developed haustoria in *Tilletia*. He relates this difference to a physiological one, pointing out that the *Ustilagos* live generally in places favorable for food collection, namely, growing points, stamens and ovaries; while the *Tilletias* are more generally leaf or stem parasites and may find these tissues less favorable for a concentrated food supply. It would be of interest to classify the species of the Uredineae upon the same basis. Has the typical and frequent haustorium been so abundantly developed in this group because the species of the Uredineae are characteristically leaf and stem parasites?

A report by Pady (52) describing the occurrence of intracellular hyphae in the orange-rust of *Rubus occidentalis* is of special interest in this connection. Pady made inoculations with a short-cycled strain of *Gymnoconia interstitialis* upon young shoots of *Rubus* and followed the course of infection from the time of inoculation to the appearance of rusted leaves the following year. The germinating basidiospore forms the usual penetration hypha in an epidermal cell but then, instead of developing intercellular branches at once, this penetration hypha develops within the host cell into a several-celled, coiled hypha. From its first, and later from its second basal cell, branches arise which penetrate adjacent cells. Each penetrating branch becomes in turn a coiled hypha from whose base other cells are infected until, by this regularly repeating unit, a hyphal complex is formed which involves many cells. Epidermal and cortical cells are thus invaded but when the phloem is reached the hyphae enter the middle lamella and continue as intercellular runners which send haustoria into the cells. By means of these runners the fungus reaches the root where it



overwinters and the following spring stimulates excessive growth of canes to form a witches broom. The change to intercellular runners begins about twelve days after inoculation; the intracellular hyphae appear to act for a time in haustorial capacity but their formation slows down as the intercellular mycelium becomes well established with haustoria; they are not found in the second season's growth. Pady thinks that this intracellular device is a temporary stage in the establishment of the systemic infection. It is important to this establishment that the mycelium reach the phloem as rapidly as possible if it is to reach the roots before the end of the growing season. Intracellular hyphae by making rapid feeding contacts could further this end. This interpretation of Pady's might apply to the *Ustilagos*. Perhaps with these smuts the presence of intracellular hyphae instead of haustoria relates to a need of rapid growth from places of infection in seedlings to places of sporulation in growing points, stamens and ovaries. Pady's interpretation indicates the haustorium as the organ of restrained feeding by the mature parasite. Lutman, suggesting that haustoria are not developed in regions of easy feeding, indicates the haustorium as an organ developed to overcome difficult conditions. Pady's work reopens the question. It may be noted that the haustorial parasite *Cercospora* upon *Vigna sinensis* (40) was said to cause little effect upon the host in its early haustorial stage but later when the attacked cells had reached the necrotic stage both inter- and intracellular mycelium was found.

Among the Uredineae lack of disturbance of the host cell by the parasite is the usual effect although occasional exceptions have been reported. Dodge (20) reports an exception in red cedar infected by *Gymnosporangium germinale*. Here the cells bordering upon hyphae, especially cells invaded by haustoria, soon show much disorganization. A nice contrast may be seen between the non-disturbing effect of the haustorium and the host cell death caused by development of intracellular spores in Pady's (50) description of teliospore development in *Calyptospora goeppertiana* in stems of *Vaccinium pennsylvanicum* and *Vaccinium canadense*. The contrast cannot be seen in Pady's figures of haustoria and teliospores in the host cells since he does not figure host cell cytoplasm or nucleus. In his text, however, he describes an early stage of infection where there are abundant haustoria in the cortical cells

and he writes of the external appearance of the stems at this stage: "The young infected shoots may readily be distinguished from normal shoots by their slightly larger size, pale green color and soft, fleshy appearance." For the later stage in which the cells contain spore initials as well as haustoria we read: "the contents of the epidermal cells begin to disintegrate and the pale green color of the stem disappears." Still later, the thick-walled teliospores in the epidermal cells give the stems a varnished, dark-brown appearance.

Not only do rust parasites cause a minimum of disturbance in the host cell but many instances have been reported of actual stimulation of the host protoplast. The gross phenomena of witches brooms in *Juniperus virginiana* infected by *Gymnosporangium nidus-avis* (66) and the huge galls of *Pinus rigida* infected by *Peridermium cerebrum* Peck (21) are familiar illustrations of hypertrophy and hyperplasy induced by rusts. The distortions produced upon *Rivina octandra* by *Puccinia Rivinae* (10) are an extreme manifestation of stimulation by the aecidial stage of a rust. However, in all such structures the infected host cells are the units where the interrelation between host and parasite needs to be observed. One is impressed by the evidence that at least for all early stages galls are the result of "the integrating activity of the cells themselves (31) rather than a disease abnormality." The galls on leaf and stem of *Sambucus canadensis* infected by *Aecidium Sambuci* are favorable for such study (58). A section shows that the enlarged mesophyll cells are surrounded by intercellular hyphae from which one or more haustoria enter nearly every cell. There is no plasmolysis and the nuclei of the host cells show phases of mitosis undergone while in contact with haustoria. Dodge (20) has made a cytological study of *Gymnosporangium myricatum*, perennial in *Chamaecyparis thyoides*, and finds there a rejuvenation of fairly old and collapsed cortical cells whenever hyphae of the advancing mycelium come near them. By this stimulation parenchyma strands burrow into the cortex like a cancerous growth.

In the matter of wall penetration the habit of the haustorium is foreshadowed even among some of the facultative parasites where the normal habit is an offensive metabolism such as Orton (48) describes for *Phyllachora graminis* upon *Agropyron repens* and

*Catacauma flabellum* upon *Pteris aquilina*. "Both are conspicuous," he writes, "for their ability to penetrate the cell walls of their hosts by dissolution." In contrast to this habit Brown and Harvey (16), after observations upon *Botrytis cinerea*, conclude that its means of penetration is purely mechanical. Abdel-Salam (2) supports this view, reporting for *Rhizoctonia Solani*, *Rhizoctonia violacea* and *Pythium* sp. upon lettuce that all these penetrate the cuticle by thin, peg-like, hyphal outgrowths which bore their way through the wall and then resume their normal thickness. This latter is the accepted method for obligate parasites. It is the habit of an haustorium to penetrate the host wall by means of a slender filament without any digestive action. Bolley (14) states of the rusts: "(they) are such perfect parasites that the hyphae pass directly through living cell walls of the host and make practically perfect fusions with the cell walls."

More distinctive even than this refinement in wall penetration by the haustorium is the lack of any puncturing or actual penetration of the primordial utricle of the host cell. Invagination of the primordial utricle which results in an increase of absorbing surface for the fungus without actual invasion of the protoplast is a generally accepted character for the haustorium. Arthur (9) states: "It is probable that in no instance does the haustorium come into organic contact with the protoplasm of the host-cell." The importance of this habit as an adaptive character lies not merely in the fact that there is no actual penetration of the host protoplast, but in the lack of disturbance of the protoplast which invagination implies. Such occasional exceptions as Dufrenoy's (23) report of plasmolysis by haustoria of *Uromyces Caladii* in *Arisaema triphyl- lum* may, I think, be due to confusion between intercellular hyphae and haustoria (59). Dodge (19) has also called attention in the case of *Diplocarpon Rosae* to the possibility of confusion between inter- and intracellular hyphae.

Dufrenoy (25) has made detailed studies of a large number of plant parasites including both facultative and obligate parasites, both mosaic and hyphal forms. He finds in all an identical host effect, that is, death resulting more or less rapidly from increased proteolytic activity. He writes: "Be it physical as evidenced in glandular cells of hairs of carnivorous plants (*Drosera*) or pathological as in cells affected by microscopic parasites or by viruses,

enhanced biochemical activity is evidenced by a division of the bulk of the involved cytoplasm into a number of slender films, spreading and forking into vacuolar material as so many partitions, which divide a large vacuole into a number of smaller ones. When the partitioning off is carried to an extreme the cytoplasm assumes a honey-combed appearance which has been described in virus-affected cells under various names but corresponds to similar structures resulting from local proteolytic activity induced by penetration of fungus haustoria, or that can even be observed in cells of tissues starving under aseptic conditions." This similarity of effect does not fit with my own observations. I agree rather with Aronescu (8) who says: "It seems that different effects and more serious ones must be expected in the cytoplasm of a host cell which has been penetrated by a hypha of a facultative parasite which does not form any haustoria, than the effects produced by a haustorium which has invaginated only the cytoplasm." In so far as my figures suggest an increase in mass of the primordial utricle I regard it as a matter of hydrolysis rather than such formations of "slender films spreading and forking into vacuolar material as so many partitions, which divide a large vacuole into a number of smaller ones."

From considerable study of plant tissues infected by rusts I am convinced that the rust parasites at least have so adjusted their demands to the metabolism of the host that a host cell surrounded by intercellular hyphae and invaded by haustoria looks, nevertheless, strikingly normal and healthy. The series of drawings shown on plate 1 is an attempt to show such conditions. They are drawn from living tissues of six different rusted plants gathered under varied conditions. Five of the six show perennial rusts. The tissues were mounted in 8 per cent sugar solution or in alcohol followed by lactophenol. The mounts were all examined under oil immersion lenses.

Figures 1 and 2 show epidermal cells from leaves of *Arisaema triphyllum*. Several *Arisaema* corms were dug up in October, 1934, washed and planted in fresh soil in the plant house. By December the plants had leaved out. Figure 1 shows a cell from the lower epidermis of a plant infected with *Uromyces Caladii* Farlow. Spermatogonia were in evidence on the leaf as soon as it appeared above the soil in December; they were fully developed

and were exuding a yellow fluid on the green leaf when the lower epidermis was stripped and mounted, inner side up, in 8 per cent sugar solution. The cell drawn lay only two cells distant from a spermogonium. The hypha which produced the haustorium belonged to the intercellular mass which ramified between mesophyll and lower epidermis and lay thus above the epidermal cell. These are conditions of heavy infection yet the host cell appears no more highly vacuolate than does the uninfected epidermal cell of figure 2 and there are no precipitation products in the vacuole.

Figures 3 and 5 are from a carnation leaf picked in February 1935 from green house stock heavily infected with *Uromyces caryophyllinus* Wint. The areas immediately around the eruptent pustules of uredospores were slightly paler than the rest of the leaf but the rusted leaves were firm and the plants were vigorous. The stripped epidermis was mounted in 8 per cent sugar solution, in inverted position. Figure 3 shows a haustorium in an epidermal cell; figure 5 shows two of the overlying mesophyll cells and hyphae which lie between these and the epidermis. The chloroplastids in the mesophyll cells were green and healthy in appearance and the cytoplasm, except where invaginated around the haustoria, was in normal position close to the wall, around the large central vacuole. The slightly different levels of the two haustorium-mother cells result in different views of the penetrating stalks of the two haustoria.

Figure 6 shows an epidermal cell from a leaf of *Potentilla canadensis* infected with *Phragmidium Potentillae-canadensis* Diet. When this perennial rust was gathered on March 16, 1935, the orange sori of uredospores were already swollen under the epidermis of the green, over-wintered leaf. The stripped epidermis was fixed in 95 per cent alcohol and mounted in lactophenol.

Figure 7 shows two parenchyma cells from an over-wintered hollyhock leaf which was fresh and green when picked on March 4, 1935, only two days after the winter snow had melted from the bed. Pustules of teleutospores of *Puccinia Malvacearum* Mont. were abundant over the leaf. The mount was a strip of epidermis in lactophenol following 95 per cent alcohol. The cells drawn lay directly below a pustule on the under surface of a vein. The penetration stalk of the haustorium in the smaller cell is drawn at the level of the intercellular hyphae. The massing of cytoplasm

around this haustorium is possibly a reaction against the parasite but there is no fragmentation of the host cell vacuole and there is the usual evidence of invagination.

Figures 4, 8, and 9 show cells of *Pyrola americana*. Figure 4 shows an epidermal cell from a strip of lower epidermis from a normal, unrusted plant. It was mounted in sugar solution. The epidermal cells of *Pyrola americana* contain chloroplasts; the cytoplasm is, for epidermal cells, unusually abundant and is frequently alveolar in appearance. Figure 9 shows two cells from the under epidermis of a *Pyrola* leaf infected with *Chrysomyxa Pyrolae* Rostr. The epidermis was mounted in sugar solution and stained with 10 per cent neutral red. This perennial rust on *Pyrola* in the spring develops uredo and teleutosori on the overwintered leaves while the new leaves of May show no rust until the following spring. The leaf, when picked on April 7, 1935, showed many uredosori under the unbroken epidermis. The cells figured lay close beside a group of sori and a heavy felt of intercellular hyphae overlay much of the inverted epidermis, projecting from under the adhering mesophyll cells. The chloroplasts in the cells figured were as deep a green as in uninfected cells. The alveolar structure of the cytoplasm in the larger cell is perhaps more noticeable than in the smaller cell of figure 4. The neutral red stain, however, indicates the normal condition of this cytoplasm; it stains the vacuole and not the cytoplasm. This cell certainly does not show the condition described by Dufrenoy (24) who says that in cells infected by rust the vacuole fragments itself in such fashion that the haustorium is surrounded by an aureole of small vacuoles, and further that, while certain vacuoles retain the neutral red stain, those in the neighborhood of a haustorium form vacuolar precipitates. At the plane figured, invagination of the host cell cytoplasm is evident. The heavily sheathed haustoria, when viewed at a higher plane than that figured, were completely veiled in the host cytoplasm, so that they appeared like inflated masses of cytoplasm. It is difficult with this fresh material to draw conclusions as to the nature of the haustorial sheaths or of such basal sockets as that in figure 7. However, in microtome sections of fixed material of *Pyrola* rust (58), the sheath takes the same stain as does the host cell wall. Most haustoria in epidermal cells of *Pyrola* are heavily sheathed and are quite generally



shrunken within the sheath. Figure 8 shows the typical haustorium found in mesophyll cells of a *Pyrola* leaf. This drawing was made from a free-hand cross section of a fresh leaf. The section was mounted in glycerine; plasmolysis in the host cell was due to conditions in the glycerine mount but it does not obscure the evidences of invagination around the haustorium.

*Chrysomyxa Pyrolae* is the only one of the rusts figured in this article which shows sheaths around the haustoria. An enclosing sheath about haustoria, varying from cup or collar to complete enclosure, is as variable yet distinctive a character for different species as the haustorial form. When it arises in connection with a thickened wall at the point of penetration of an haustorium it appears to be a host cell product. Smith (60) describes such a formation for *Erysiphe graminis*. Tai (65) gives almost the same explanation for the interaction of *Juniperus chinensis* and *Gymnosporangium Yamadae-Miyabe*. The host cell is stimulated by the penetrating haustorium to build a protective sheath but there is a limit to this defensive activity and then the haustorium digests away all but a thin investing membrane. Moss (46) finds in the Pucciniastreae all stages of the sheath from absence to complete enclosure. He gives a generally accepted view in the statement: "It appears that the sheath is laid down by the surrounding protoplasm of the host in response to a stimulatory action of the haustorium. Undoubtedly this constitutes a defense on the part of the host cell because the encapsuled haustoria are in disorganized condition." The sheathed haustoria of *Chrysomyxa Pyrolae* shown in figure 9 are evidently disorganized but according to other observations (58) sheathing does not by any means result always in disorganization. Many haustoria of both *Puccinia Sorghi* Schw. and *Aecidium claytoniatum* Schw. are plump, nucleated structures within heavy sheaths. It would contribute toward an explanation of the significance of the sheath if the development of the mycelium in leaves of *Pyrola* were followed to determine whether the sheathed haustoria in epidermal cells are formed early or late in the spreading infection, whether they are the result of a weakening parasite, of a host especially vigorous at the beginning of leaf infection, or of a host whose epidermal cells are more able than other leaf cells to build sheaths because of their habit of building thick walls. Figures 3

and 9 show the characteristic thick walls of epidermal cells of *Pyrola*. There remains also the unsolved problem of thickenings on the haustorial stalk not in connection with the host cell wall such as I have reported for *Puccinia Sorghi* (58). Here again *Pyrola americana* offers especially favorable material because of the abundant cytoplasm in its cells. In figure 9 the stalk of the haustorium in the smaller cell is seen in external view instead of in section as in the larger cell. Across the stalk may be traced the line of both plasma membrane and tonoplast. Are these structures of the cell especially potent in the laying down of wall substance? The effect of a sheath upon the feeding of the enclosed parasite is also open to debate. The inference seems a safe one which Aronescu (8) makes for *Diplocarpon Rosae* that a sheath reduces the absorbing surface of an haustorium. To this statement I would add: "If cellulose walls allow the passage of food through them into intercellular hyphae, then cellulose sheaths can at best merely lessen the amount absorbed" (58).

The remaining figures on the plate are studies of *Aecidium punctatum* Pers. and its host, *Hepatica acutiloba*. On February 12, 1935, several *Hepatica* plants were lifted from under two feet of snow and brought into the plant-house. On February 16 the winter buds had opened and blossoms had appeared. On one plant leaves instead of blossoms were pushing up. This indicated rust as *Aecidium punctatum* induces sterility in *Hepatica*. The leaf picked for study was only just above the soil and its lobes were closely rolled but the under surface was dotted with spermogonia, each capped with a drop of pale yellow fluid. The epidermis was stripped off and mounted inner side up in 8 per cent sugar solution. Figures 11 and 13, drawn from this tissue, show the dense contents and relatively large nuclei of the cells from this rolled leaf and show the line marking the surface of the invaginated cytoplasm around each stalked and coiled haustorium. Figure 10 shows a cell from the lower epidermis of an older leaf from the same plant. This leaf was cut on February 20 after it had expanded into the stiff, long-petioled blade which is characteristic of rusted *Hepatica*. The spermogonial liquid had deepened to orange-yellow and aecidia showed as white spots beneath the epidermis. The epidermis was mounted in 8 per cent sugar solution. The figure shows a larger host cell vacuole and a more elaborately

coiled haustorium than those in the younger leaf. Both of the infected *Hepatica* leaves were thickly studded with spermogonia and practically the whole leaf area between lower epidermis and mesophyll was crowded with intercellular hyphae from which haustoria entered the cells above and below. Figure 10 shows an overlying hypha from which a haustorium arises; figure 13 shows in cross section an overlying hypha from which a haustorium enters one of the cells. Figure 12 shows cells from the lower epidermis of a leaf of an unrusted plant. This leaf, following the regular habit in *Hepatica*, developed after the plant had finished blooming but it was, when picked, in the same closely rolled stage as the one from which figures 11 and 13 were drawn. A comparison of figure 12 with figures 11 and 13 proves that the infected host cells are close to normal in appearance. The cells of the infected leaf show the same conditions of nuclei, cytoplasm and vacuoles as those of the uninfected leaf of the same age. Variations in these cell structures are evidently physiological and not pathological effects.

Only second to the question of invagination, in any discussion of haustorial host cell relations, is the question of the reaction of the host nucleus. I am repeatedly impressed with the very normal spherical shape and normal size of the host nucleus in the majority of infected cells. In a set of forty-three drawings of cells from corn tissue (58), of varying age and varying degrees of infection with *Puccinia Sorghi*, twenty-three of the cells showed nuclei; only four of these nuclei were abnormally large; only two of these were lobed. I have found more striking instances of enlarged, lobed nuclei in galls upon *Sambucus canadensis* infected with *Aecidium Sambuci* Schw. and in leaves of *Claytonia virginiana* erumpent with spermogonia and aecidia of *Aecidium claytoniatum* Schw. That these abnormal shapes do not of themselves predicate ill health has been pointed out by Goldstein (29) who finds that hypertrophied, irregularly lobed nuclei occur normally in healthy plants, in special organs of nutrition, and associated with certain phases of nutritional and secretory activity.

Contact between haustorium and host nucleus seems, both from reports in the literature and from my own observation, to be a variable phenomenon but a large majority of investigators report that haustorium and host nucleus are habitually or frequently in

contact. Cunningham (18) goes farther than this stating for the rusts: "when entry is effected the haustoria invariably grow in the direction of the host nucleus." Dodge (19), reporting upon *Diplocarpon Rosae*, indicates the opposite view in the statement: "No evidence has been found to indicate any particular attraction or repulsion as to the relation of haustorium and host nucleus." As to which member brings about the contact and for what purpose there is little unanimity in the reports. They include purposes of defense and offense by the nucleus and a furtherance of absorption by the haustorium. The latter purpose seems to fit the specially adapted haustorium but it presupposes active growing of the haustorium toward a movable part of the protoplast. Allen (6) makes a case against this in her observations of *Puccinia glumarum* on *Bromus marginata* and *Triticum vulgare* since in the 82 per cent of contacts reported in her observations of this rust many of the haustoria were located at the end of a cell three or four times as long as the haustorium. The contact between the two can have been achieved, she thinks, only by the motion of the nucleus toward the haustorium.

Probably the most delicate indicator of health in a photosynthetic plant is the chlorophyll. Hence, by this indicator one can most effectively recognize the delicate adjustment of the haustorial parasite to the host cell over against the devastating action of the non-haustorial facultative parasite. Two instances of this devastating action follow: a fungus upon *Zostera marina* thought by Peterson (55) to be *Ophiobolus maritima* blackens the infected cells and causes finally the death of the leaf; *Fusicladium saliciper-dum* (15) blackens infected cells of willow leaves in ever widening zones until the whole leaf may be affected. Brooks and Walker are undecided whether this death of cells beyond the infected zone is due to products which diffuse from the cells first killed or to toxic secretions of the fungus itself. Mosaic diseases illustrate a less destructive effect but Nelson (47) states: "Chloroplast degeneration seems to be one of the microscopic symptoms of mosaic disease in many plants." Even many of the haustorial fungi show disturbing effects upon chloroplasts. Weston (71) states that the systemic infection of *Sclerospora graminicola* on Everglade millet shows itself chiefly in the development of pallid, yellowish markings in the dark green tissue of the leaf. R. E.

Smith (61) reports disorganization of the chloroplast in cells of asparagus when entered by haustoria of *Puccinia Asparagi*. He thinks the immediate collapse and clumping of the chloroplasts is due to some soluble substance, poisonous or enzymatic, excreted by the haustoria. The hosts of aecidial infections are frequently chlorotic. For example, plants of *Houstonia coerulea* infected with *Aecidium houstoniatum* are markedly etiolated (58). Dufrenoy (22) states that galls formed on chlorophyll tissue if not colored by anthocyanin are usually colorless. He reports galls upon leaves of *Asphodelus subalpinus* infected by *Puccinia Asphodeli* in which there is an exaggerated activity of chlorophyll concomitant with the growth of the young galls but a discoloration of adult galls concomitant with the development of enormous tannin vacuoles and an accumulation of reserve starch. In still another case of an aecidial infection Reed and Craybill (57) report that the palisade cells in the rust lesions of *Gymnosporangium Juniperi-virginianae* contain carotin and erythrophyll but no chlorophyll. I can endorse reports of the yellowing effects of this rust. A pasture grown up to thorn apple trees drew my attention one July because practically all the leaves were spotted with orange. The orange coincided with thickened leaf areas which bore the roestelia of *Gymnosporangium Juniperi-virginianae* Schw. Cytological examination of the leaves showed that the space between the upper and lower epidermis of the infected areas was packed with enlarged cells making a cushion four times the width of the uninfected, green portions of the leaves. The cells of the infected area were abnormal only in size. Their chloroplasts, instead of being disintegrated as the yellow color might lead one to expect, were larger than those in the green areas beyond the infection and were swollen with grains of assimilation starch. Apparently the photosynthate was drawn from its place of manufacture to the infected cells. Such a drain must eventually lead to disorganization even with a highly adapted parasite. Any observer of rusts knows the starvation and dessication effects which attend sporulation in heavy rust infections of the cereals. Cunningham (18) states: "The annual losses to agriculture caused by 'Rusts' have been computed at upwards of £100,000,000 sterling in cereal crops alone."

In contrast to the above effects, Lamb (39) writes of *Puccinia Prostii* infecting *Tulipa sylvestris* var. *odorata* that the pustules

cause no discoloration of the surrounding leaf tissue. The many examples from the Uredineae of effects similar to that of *Puccinia Prostii* seem to warrant treating the Uredineae as the climax type in the above series. For the rusts which I have shown in plate 1 there is no need to qualify the statement that there is no discoloration around the pustules. The winter rosettes of *Potentilla canadensis* on the lawn catch one's eye because of the orange pustules on the green leaves. The green surfaces of *Pyrola* leaves in the spring woods make a striking contrast to their orange under surfaces where uredo pustules have destroyed the epidermis. These leaves wither as the new leaves develop but apparently not much sooner than those of uninfected plants. The spring leaves of *Hepatica*, metamorphosed into stiff, long-petioled blades by the rust, are green even when their under surfaces are covered by the flower-like aecidial cups. These infected leaves, however, wither by mid-summer and are replaced by a cluster which develops without any sign of rust and persists over the next winter as do the leaves of uninfected plants. *Chrysomyxa Pyrolae* and *Aecidium punctatum* are both outstanding examples of restraint on the part of the parasite toward the hosts in which they perennate but with the difference that *Chrysomyxa Pyrolae* comes to fruition on the over-wintered leaves while *Aecidium punctatum* fruits on the new spring leaves.

More striking even than the lack of discoloration in rust-infected leaves is the intensification in color and the lengthened life of chloroplasts found in many rust infections. "The basic fact here is (58) of course the prolongation of the life of the entire cell, and not merely the persistence of the green color." This so-called "green island" phenomenon is a decisive illustration of a longer life given to infected cells under conditions of adversity to the host plant. From this standpoint both "green islands" and gall formations are stimulation effects which differ only in degree. Wingard (72), illustrating bean strains resistant to *Uromyces appendiculatus*, shows an island of green tissue in the discolored flecks due to abortive uredosori. Here the cells in the center of the infection seem stimulated to more vigorous conditions than the cells at the border of the flecks which are in the early stages of invasion by the rust. A study of leaves (58) made in September from a field of Golden Bantam corn infected with *Puccinia Sorghi*



shows "green islands" on the lower leaves. Here the condition of adversity was the drying incident to the normal dying of the older leaves at this late season. Green-bordered sori are figured abundantly in a yellowing but still succulent leaf; they are present in a nearly dry leaf; and a few persist in a dry, brown, basal leaf. Mains (43) explains this phenomenon as due to a levy made by the infected cells upon the uninfected cells surrounding them. Thus the cells of the infected area are supplied with water and are kept in fair health long after the uninfected cells have dried up.

A comparison of this phenomenon with that of the "shot-hole" effect indicates again the higher adaptation of the Uredineae. In the rust infection there is little or no defense reaction by cells surrounding an infection whereas Higgins (32) explains the "shot-holes" in *Prunus virginiana* infected by the haustorial fungus *Cylindrosporium* as due to a defense action set up by cells bordering the infected spot. These become an abscission circle of heavy-walled cells which cut off water from the "spot." Then the infected cells shrivel and drop out.

The same contrast between the poorly and the highly adjusted host-parasite complex may be seen with the one rust in a resistant and in a susceptible host. Allen (4) draws a picture "of excellent congeniality" for *Puccinia triticina* physiologic form 11 on Little Club wheat. "The fungus," she says, "attains its maximum development. . . . The host tissues live, their cell walls (except in the guard cells at the stoma of entry) are not damaged, and the cell contents show a minimum of disturbance or impoverishment." Her description of the same rust form on the resistant Malakoff wheat (5) corresponds to the effects of an offensive, facultative parasite. The first host cells invaded collapse and die promptly. Many of the rust fungi die also; as the survivors succeed in entering host cells and forming haustoria the host reaction becomes milder and a few of the mycelia may bear small uredinia. There is as with *Cylindrosporium* a change beyond the fungus—cells bordering the infected area show irregular, swollen walls.

In this description of the reactions observed in a resistant host one notes emphasis upon the haustorium as the organ which effects a fine adjustment between host and parasite. Hull also (34), discussing types of resistance to *Puccinia Sorghi* shown by *Zea Mays*, says that if the growth of the invader in a resistant host is

rapid enough to make haustorial contacts with host cells before the latter have time to establish any antagonistic response there is then a perfect equilibrium between host and fungus and henceforth formation of haustoria produces no noticeable reaction in the host cells. Such descriptions indicate "the host cell as the place where resistance is expressed, and the attempted penetration of a haustorium as the act by which resistance is stimulated" (58).

The literature offers in increasing number theories concerning the nature of plant resistance to fungus parasites. Wingard (72) concluded a study of rust-resistant beans as follows: "Rust resistance in beans can best be explained on the basis of the hypersensitiveness of the host to the parasite. The immediate destruction of the host tissue results in the death of the parasite because the rust fungus is an obligate parasite and cannot survive in dead tissue." In this sense resistance arises from lack of adjustment. The fungus corresponds to a facultative parasite except that it cannot live upon dead tissue. Allen's description, already quoted, of rust in resistant Malakoff wheat is an illustration (5). Allen states also (3) that immunity is due to definite antagonistic chemical interactions between host and parasite. Stakman early concluded (63) that the problem as to the instruments of immunity called for biochemical investigations. Ezekiel (27) has carried out such an investigation by testing the growth of urediniospores in hanging-drops of materials extracted from normal as well as infected wheat tissues. He found that the extracts affected the growth of physiologic forms of *Puccinia graminis tritici* in a manner diagnostic of the resistance of the host to the various physiologic forms. He concluded: "These materials in the plant tissues presumably explain the phenomenon of physiologic resistance to stem rust." In one experiment Ezekiel found that differentiation was greater with extracts from inoculated plants than in extracts from normal plants and suggests that antibody activity may be, but is not necessarily, involved. This suggestion points out a problem on which much work needs to be done. I think it likely that we are too apt to explain a lack of adjustment between fungus and host as due to toxins. It is possible that the answer to the problem as to the instruments of immunity to an adaptive parasite lies neither in toxins nor in antibodies but, as Mains (44) has suggested, in the normal metabolism of the various hosts. Con-

versely then susceptibility would be brought about by a fungus whose metabolism has been closely adjusted to and linked with the metabolism of a host. Wellensiek (70) really subscribes to this theory when he writes that in corn "resistance to *Puccinia Sorghi* is the expression of starvation of the parasite."

It is the very fact of obligateness, that is, of a high degree of specialization in hosts, which has furthered the breeding of rust-resistant plants. Aamodt (1) points out: "Narrow specialization of the parasite with definite host reactions for the different forms has made it possible to analyze the pathogene and to study its host relationships in such a way as to be of considerable assistance to the plant breeder." He contrasts this condition with the greater difficulty met with in breeding varieties resistant to the foot-rot organisms such as *Ophiobolus*, *Helminthosporium*, and *Fusarium* because of the lack of definite and characteristic reactions of the hosts to the different pathogens and their physiologic forms, and to the wide parasitic range of physiologic forms in their ability to attack different varieties. The rusts, of course, are not alone in this class of physiologic specialists but that they are eminent members of the class the familiar history of *Puccinia graminis* proves. Stakman wrote in 1926 (64): "*Puccinia graminis* long ago ceased to be merely *Puccinia graminis*; it first became *Puccinia graminis tritici*, *Puccinia graminis secalis*, etc. Now it has even become *Puccinia graminis tritici*, physiologic forms 1 to about 50, inclusive." The list has grown. In February, 1935, Humphrey (35) reports: "Over a hundred distinct parasitic strains or physiologic forms of the stem rust of wheat have been discovered, more than 50 of which occur in the United States." To date he adds (36): "a few additional physiologic forms have been included in the list, bringing the total number up to something like 152, not all of which occur in the United States." Such a record supports Orton's statement (49): "all the evidence available at the present time appears to point to the conclusion that within the Uredinales the trend is toward increased physiological specialization." This record for *Puccinia graminis tritici* is, of course, the result of intensive work carried on largely because *Puccinia graminis tritici* is an enemy to agriculture. It at least indicates what a rust can do when its host is a plant which offers many varieties. This raises another side to the problem of specialization. Jackson (37) writes: "To a

close student of the rusts it is evident that the host has been a very important factor in the evolution of species in this group. As the higher plants have gradually developed during past ages, the rusts parasitic upon them have likewise undergone development."

One returns from a consideration of the rusts to de Bary's classification of fungi with a fuller understanding of the term obligate parasite. The condition for the rusts is summarized by Hotson (33) as follows: "The Uredinales include a group of very highly specialized fungi which are obligate parasites. As far as is at present known they can grow only on living plants (the hosts) and when these are killed the rust dies. Many rusts are so highly specialized that they confine their activities to relatively few species of the host, some to a single species, others to different species in the same genus; rarely do they produce the same spore-forms on more than one host family." We have found that specialization is a corollary to obligateness; the high degree of specialization has been effected, I believe, by the elaborate development of the haustorium. The result is a condition which de Bary (13) places close to that of the lichens on the basis of the limited host accommodation on the part of both rusts and lichen fungi. While the mutualistic nutrition of the lichens is, of course, not paralleled in the rusts the habits of the majority of rusts indicate a restraint in feeding and a reduced or entire absence of toxic action upon the host by the parasite. In the case of "green islands" one might perhaps call the relation between the rust and the host cells temporary mutualism.

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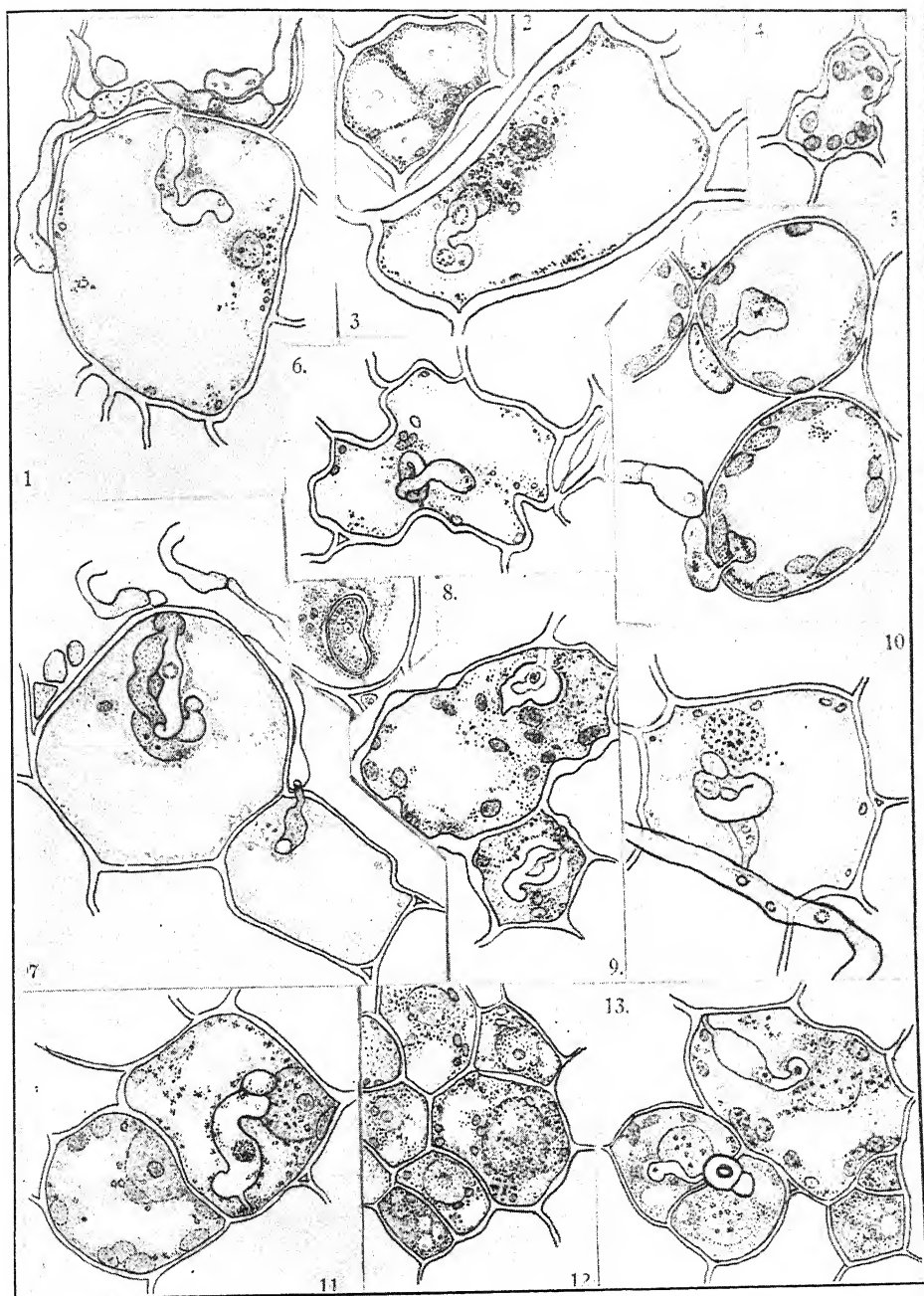
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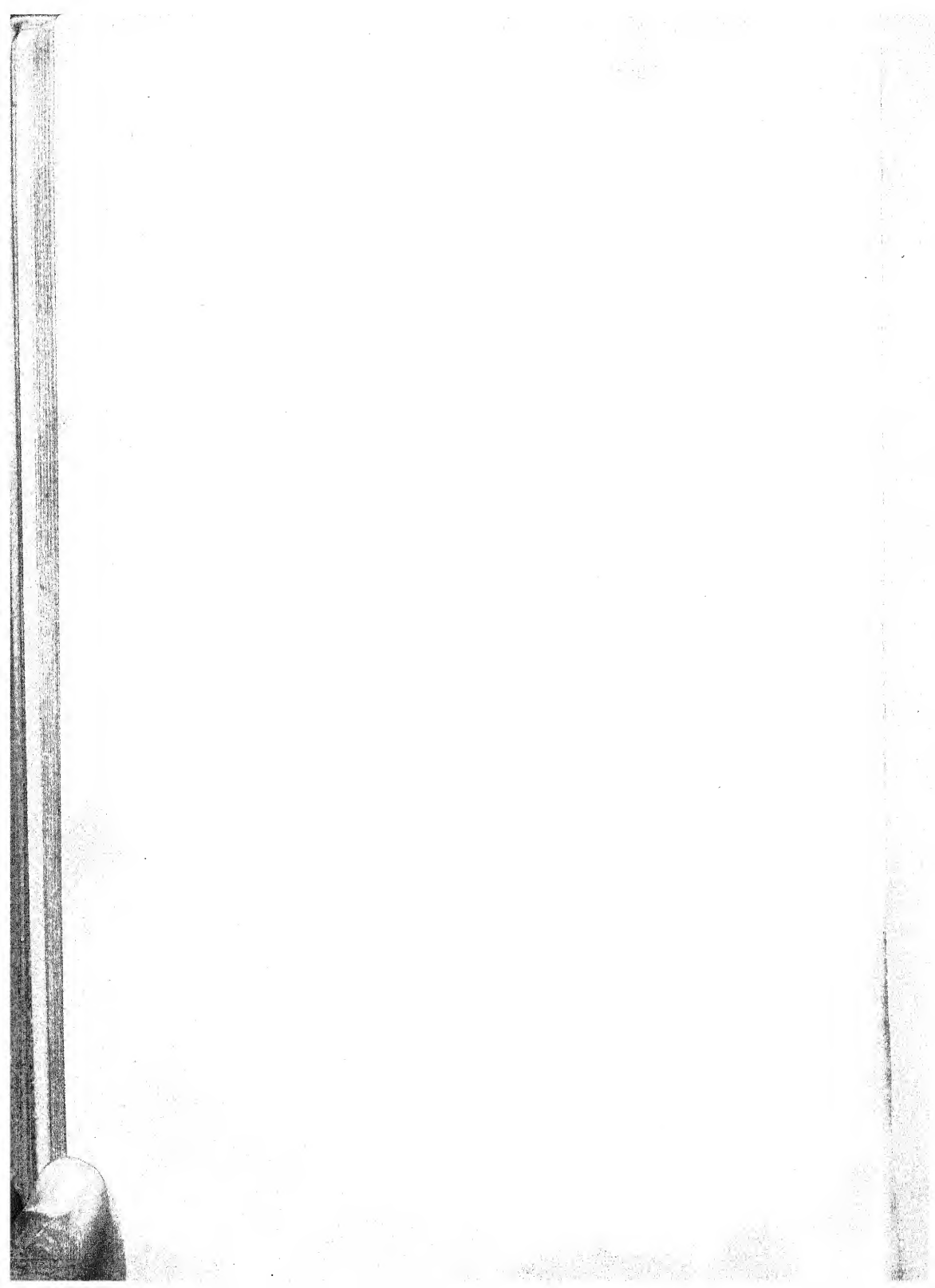
## DESCRIPTION OF PLATE

The figures of Plate I were drawn with the aid of a camera lucida from free-hand sections of fresh leaves. A Spencer microscope was used with an apochromatic objective, 4 mm., and ocular, 10 X, giving an approximate magnification of 650 for the outlines. For details of the drawings a 1/12 inch oil immersion objective and an ocular 10 X were used.

## PLATE I

- Fig. 1. Cells from lower epidermis of a leaf of *Arisaema triphyllum* infected with *Uromyces Caladii* Wint., showing intercellular hyphae and a haustorium. Mounted in 8% sugar solution and stained with 10% neutral red.
- Fig. 2. Cell from lower epidermis of a leaf from an uninfected plant of *Arisaema triphyllum*. Mounted in 8% sugar solution and stained with 10% neutral red.
- Fig. 3. Epidermal cell from a leaf of *Dianthus caryophyllus* infected with *Uromyces caryophyllinus* Wint. Mounted in 8% sugar solution.
- Fig. 4. Cell from the lower epidermis of a leaf of an uninfected plant of *Pyrola americana*. Mounted in 8% sugar solution.
- Fig. 5. Mesophyll cells adhering to the strip of epidermis used for figure 3, showing haustoria and the hyphae which lie between epidermis and mesophyll. Mounted in 8% sugar solution.
- Fig. 6. Cell from lower epidermis of leaf of *Potentilla canadensis* infected with *Phragmidium Potentillae-canadensis* Diet., showing a haustorium. Fixed in 95% alcohol and mounted in lactophenol.
- Fig. 7. Parenchyma cells of a hollyhock leaf infected with *Puccinia Malvacearum* Mont., showing haustoria and intercellular hyphae. Fixed in 95% alcohol and mounted in lactophenol.
- Fig. 8. Detail of mesophyll cell from leaf of *Pyrola americana* infected with *Chrysomyxa Pyrolae* Rostr., showing haustorium. Mounted in glycerine which caused plasmolysis.
- Fig. 9. Cells from lower epidermis of leaf of *Pyrola americana* infected with *Chrysomyxa Pyrolae* Rostr., showing sheathed haustoria within invaginated cytoplasm. Mounted in 8% sugar solution.
- Fig. 10. Cell from lower epidermis of a half grown leaf of *Hepatica acutiloba* infected with *Aecidium punctatum* Pers. A coiled haustorium arises from an intercellular hypha which lies above the cell. Mounted in 8% sugar solution.
- Fig. 11. Cells from the lower epidermis of a young, rolled leaf of *Hepatica* infected with *Aecidium punctatum* Pers., showing a haustorium. Mounted in 8% sugar solution.
- Fig. 12. Cells from the lower epidermis of an uninfected leaf of *Hepatica acutiloba* at the same stage as the leaf of figure 11. Mounted in 8% sugar solution and stained with 10% neutral red.
- Fig. 13. Cells from the lower epidermis of the same leaf as that of figure 11, showing haustoria and cross sections of intercellular hyphae. Mounted in 8% sugar solution.





# LIGHT<sup>1</sup> AS AN ECOLOGICAL FACTOR AND ITS MEASUREMENT

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Light is one of the most important factors in the growth of plants and also one of the most difficult to study. In order to understand many of its specific effects, workers have resorted to experiments in small chambers using artificial light, and in so far as possible, artificial climate also.<sup>2</sup> Such experiments are invaluable for discovering the fundamental influences of light and form the basis upon which ecological data can be interpreted.

During the past 15 years, another method of studying the light relations of plants has been widely used. This consists of measuring the daily course of photosynthesis of plants growing in natural habitats and in correlating the photosynthetic rate with light, carbon dioxide supply, stomatal aperture, temperature and other factors. These studies, together with those on the rate of growth of plants in various habitats, are leading us more and more toward an understanding of the reaction of plants to their environment.

The variations in light conditions to which plants are subjected, may be grouped into two classes:

1. Those variations which are caused by differences in latitude, altitude and climate.
2. Those variations which are caused by local obstructions such as forest canopies.

Both classes of variation include differences in the intensity and quality of the light.

## *Light-Climate*

The term "light-climate" (*Lichtklima*) was introduced by German workers (132) to designate the light conditions prevailing in

<sup>1</sup> The term "light" will be used in this paper as synonymous with solar radiation received at the surface of the earth unless otherwise qualified.

<sup>2</sup> In the Division of Biology and Agriculture of the National Research Council, a subcommittee on Survey of the Committee on Radiation is preparing, with the collaboration of a large number of individual workers, a comprehensive report on the influence of radiation on living organisms which will also include methods of measuring radiation. In this paper the writer is limiting the treatment mainly to the ecological phases which will not be included in the above mentioned report.



various habitats. The writer prefers to restrict the use of this term to the variation of class 1; however, the same term may be used for both by designating class 1 "Macro-light-climate" and class 2 "Micro-light-climate."

The intensity and quality of light received at any point on the earth's surface depends upon the solar constant,<sup>3</sup> the distance of the sun from the earth, and the absorption and diffusion of light by the atmosphere. From the standpoint of the plant ecologist and plant geographer, variations in the solar constant are insignificant. However, it is important to know the maximum intensities of light which may be received at the earth's surface, and how these compare with the values reported by investigators in ecology and physiology. The highest values of solar radiation ever measured at the surface of the earth on high mountains are about 1.75 gram calories per square centimeter per minute (76). This value corresponds to a maximum of approximately 12,000 foot candles (75). Values at sea-level are rarely above 1.5 gram calories per square centimeter per minute, or 10,000 foot candles. These figures should be kept in mind in reading some of the earlier ecological studies of the light factor, since investigators have reported values of 17,000 to 20,000 foot candles, which obviously do not occur in nature.

The distance of the sun from the earth, which is greatest in early July and at a minimum in early January, does cause an appreciable influence on the intensity at the earth's surface. For the same solar zenith distance,<sup>4</sup> the intensity is about 10 to 15 per cent. higher in the winter.

Even greater variations in the amount of light received at the earth's surface are caused by the influence of the atmosphere. Particles of dust, smoke and even gas molecules cause a scattering of light. This scattering is more pronounced with the shorter wavelengths, that is, the blue region of the spectrum. Approximately one half of the light so scattered reaches the earth as skylight. Gases in the atmosphere have definite absorption bands which also change the quality of light received. Water vapor in the air ab-

<sup>3</sup> The solar constant is the intensity of solar radiation outside the earth's atmosphere for the sun in the zenith (directly overhead) and at its mean distance from the earth. It is equal to 1.94 gram calories per square centimeter per minute.

<sup>4</sup> Solar zenith distance is the angle at the observer between lines to the sun and to the zenith.

sorbs a great deal of the long wave-lengths, particularly infrared, while ozone absorbs ultraviolet. The amount of both absorption and scattering which occurs increases with the air mass traversed, or the secant of the solar zenith distance. Absorption by the atmosphere is most important in reducing the intensity of light, while diffusion is most important in changing the energy distribution. Consequently, in winter, due to the greater diffusion caused by an increased solar zenith distance, a higher percentage of red light and a lower percentage of blue reaches the earth than in summer.

One of the most important factors causing variations in the light received is the humidity of the atmosphere and the amount of cloudy weather. Arid regions have higher hourly values and higher yearly totals than humid regions of the same latitude.

Similar variations occur between tropic and arctic regions. During the growing season the amount of light received during the average day in the tropics is not necessarily greater than the amount received in temperate regions, and only slightly greater than the amount received in the arctic regions (93). The hourly maxima are, of course, greater in the tropics than in the arctic regions. Also, the yearly totals are higher in open country than in smoky cities. An excellent summary of light-climate is given by Kimball (76) which has been freely drawn upon in the above discussion. An earlier but less comprehensive summary of light-climate is given by Rübel (132).

In the above discussion little has been said about variations in the radiation intensity during the course of a single day. The effective energy which reaches any point on the earth's surface at any instant is composed of skylight and the vertical component of direct sunlight which varies directly as the cosine of the solar zenith distance. Consequently for any station we have a continual change in both the intensity and quality of solar radiation received from hour to hour, from day to day, and from season to season.

#### *The Effect of Light-Climate on Plants*

Variations in light caused by differences in latitude, altitude and climate naturally affect rate of photosynthesis and growth of plants. Since during the growing season the difference in light between tropic and temperate regions is not very great, we might

expect that photosynthesis would proceed at approximately the same rate in both regions. Stocker (165) has found this to be the case. Plants in the tropical rain forest assimilated carbon dioxide no more rapidly than plants similarly exposed in the temperate regions. Studies by Müller (111) and by Kostytshev, Tschesnokov, and Bazyrina (89) have shown that in arctic regions plants may assimilate carbon dioxide continuously and that the total amount assimilated during the 24-hour arctic day may be almost as great as would be assimilated during a summer day in temperate regions.

Instead of being the limiting factor, light intensity in the arctic may be excessive. Lipmää (93) has pointed out that many arctic plants develop the same type of protective features against intense light that are characteristic of desert plants in temperate climates. In other words, xerophytes and mesophytes occur in the arctic regions as well as in warm regions. Arctic xerophytism is probably accentuated by the low moisture supplying power of cold soil which may be of more importance than physical dryness of the soil (29).

The exposure of tomato plants and certain others to 24-hour illumination in temperate regions, either continuous artificial light or daylight supplemented by artificial light of high intensity, results in severe injury and death (8). The injury observed is diminished if the plants receive some daylight or if the Mazda lamps are supplemented by mercury arcs. The unfavorable effects observed may be due to the unfavorable quality of artificial light, since Darrow (31) has shown that tomatoes and other plants will grow and thrive under continuous daylight of the arctic regions.

Many evergreen plants are able to perform photosynthesis in winter at a sufficiently rapid rate to more than balance the carbohydrates used up by respiration, even at temperatures near freezing (183, 73, 35). The total amount of photosynthesis which occurs in winter, however, is probably of minor importance since during the winter months profound changes occur in the arrangement and size of the chloroplasts, which are accompanied by their inactivity (122, 59, 73).

#### *Light in Local Habitats*

Plants which grow in the forest and those which grow under water are subject to light which has undergone additional modifi-

cations. The intensity of light available for plants growing under water below a depth of one meter, decreases more or less uniformly with depth (18). Water absorbs energy in the infrared and red region to a much greater extent than in the blue, hence plants in clear water receive a relatively large percentage of light within the region 440 to 580 m $\mu$ <sup>5</sup> (19, 13, 27). Particles suspended in the water cause a scattering of the blue rays more than the red; therefore, light which has passed through turbid water tends to have a lower percentage of blue than that passing through pure water (27). Consequently, both the quality and intensity of light available for submerged plants is dependent on the depth at which the plants are growing and the turbidity of the water.

A number of studies have been made on the rate of photosynthesis of submerged plants. Obviously, plants cannot live indefinitely in light intensities too low to permit sufficiently rapid photosynthesis to balance the carbohydrates used up in respiration. The depth at which the compensation point occurs naturally depends on both the species and amount of light available to the plants. Bourn (21) found that at least four per cent of radiant energy was required for the growth of *Potamogeton pectinatus*. Clarke and Oster (27) found that the point at which photosynthesis just balanced respiration for certain phytoplankton occurred from 7 to 20 meters in turbid water, and at 30 meters in clearer water. The total radiation intensity was about three per cent in each case. Schomer (137) found that the optimum depth for photosynthesis in lakes of northern Wisconsin was at the surface on cloudy days and at a depth of about five meters on fair, bright days. The compensation point was between 10 and 15 meters. In the more turbid lakes these depths were less.

For each type of water plant there seems to be a more or less optimum intensity for photosynthesis (109, 63, 78). The brown and green algae require higher light intensities for a photosynthetic balance than the red algae (175, 109). Seybold (143) has shown

<sup>5</sup> Millimicrons. One millimicron = .000001 millimeter or 10 Ångstrom units. The solar spectrum may roughly be divided as follows:

Wave-lengths 290-400 m $\mu$  ultraviolet  
401-492 m $\mu$  blue  
493-535 m $\mu$  green  
536-620 m $\mu$  yellow  
621-720 m $\mu$  red  
721- m $\mu$  infrared.

that the red algae absorb a greater percentage of blue light than either the green or brown algae, which may account in part for their ability to live at greater depths.

### *Light in the Forest*

The light available for growth of plants on the forest floor may be greatly reduced in intensity. Frequently this reduction amounts to as much as 90 to 99 per cent (11, 32, 49, 52, 132). Moreover, the intensity fluctuates almost continuously due to movements of the foliage. The intensity in sunflecks may be as high as 30 to 40 per cent of full sunlight, while in the shadow the light may be as low as two to five per cent. Where an understory of either young growth or shrubs occurs beneath a closed canopy, the light intensity is generally around 20 per cent above this second story, but may be reduced to from one to five per cent below it (70, 149).

Not only is the intensity of light in the forest lower than that in the open, but the quality is also changed due to differential absorption and reflection by leaves. Knuchel (79) found that the change in light quality under canopies of needle-leaved trees was slight but beneath broad leaved canopies the light was relatively higher in percentage of green. He studied the spectral transmission of leaves and found a much higher transmission in the green region than in the blue or red regions of the spectrum. A large number of studies on the reflection, transmission and absorption of leaves have been carried out since then which confirm these findings (11, 12, 32, 62, 119, 135, 138, 139, 140, 141, 142, 143, 154, 155, 160, 167).

### *Light Requirements as to Intensity*

It follows from the discussion of light-climate, that light intensity during the growing season is adequate for satisfactory growth of plants in all latitudes from the tropics to the arctic regions. In fact, the intensity may be so high as to cause injury to plants, particularly to young seedlings (57, 82, 92, 174).

In desert regions where light intensities are particularly high, plants actually make use of only a small fraction of the light available. It has recently been shown that when leaves are exposed under conditions favorable for rapid photosynthesis, the rate of photosynthesis tends to decrease with increasing time, due to an accumulation of carbohydrates in the leaves (66, 90). This is spoken of as "solarization" of leaves.

A number of German and Russian workers have studied the rate of photosynthesis of plants in natural habitats, particularly of desert plants (20, 56, 83, 84, 85, 86, 87, 88, 110, 136, 156, 184). These workers have found that the daily rate of photosynthesis shows marked fluctuations. Frequently a maximum occurs around 9 to 10 o'clock in the morning and sometimes even earlier. A minimum occurs about noon when some plants may even give off carbon dioxide while a secondary maximum in the afternoon is not uncommon.

Several factors probably influence the rate of photosynthesis under desert conditions so that it does not attain as high values as would occur in the more favorable habitats. High temperature and insufficient moisture supply are probably most important while solarization of leaves is probably secondary. Miller and Burr (107) have shown that a number of plants will give off carbon dioxide in light of 2000 foot candles when exposed to temperatures of 35° to 37°C. Measurements of stomatal aperture show them to close in the light when moisture supply becomes low. Stålfelt (158) and others (50) have shown that the hourly rate of photosynthesis follows closely the size of the stomatal aperture. Hence both photosynthesis and transpiration may decrease under conditions of high light intensity and limited moisture supply (20, 26, 136).

Espino and Pantaleon (36) have found that under conditions of high light intensity, plants exposed to full sunlight in the morning and shaded in the afternoon, produce much better growth than those exposed to sun all day, or those exposed to the afternoon sun only. This is in agreement with the results on photosynthesis.

Several attempts have been made to correlate the rate of photosynthesis with measurement of external factors, including temperature, humidity, transpiration, evaporation, carbon dioxide concentration of the air and light intensity (20, 32, 58, 109, 136). Most of these have not met with particular success. Since photosynthesis is dependent on a number of external factors, first one then another may be limiting, and this appears to be the case when attempts at correlation are undertaken. During part of the day, photosynthesis may be correlated with light. Later on in the day it may be correlated with transpiration, when this

factor is closely related to stomatal aperture, and under certain conditions it has been found to be correlated with carbon dioxide concentration.

While Beljakoff (16) has found that the rate of photosynthesis should change rather gradually during the day unless other factors fluctuate violently, and questions whether the rapid fluctuations reported by Kostichev, Rosumov and Tschesnokov (87) might not have been due to inaccuracies in their methods, the work of other investigators seems to indicate that under natural conditions, light intensity, carbon dioxide supply, water supply and many other factors affecting photosynthesis, are subject to violent fluctuations and that these fluctuations cause similar variations in photosynthesis.

Although there has been considerable controversy over the exactness of Blackman's theory of limiting factors in photosynthesis, there can be no doubt of its application when broadly interpreted (67). Often two or more factors may be near limiting values (156 a) so that changes in these together with rapid changes in internal factors may account for the wide fluctuations in photosynthesis which have been reported.

The increase in dry weight of plants growing under natural conditions is closely associated with their rate of photosynthesis (32, 58). For best development, most plants require full sunlight, provided other factors are not limiting. With increasing light intensity, there is generally an increase in rate of growth up to 50 or 100 percent of full sunlight (146). This appears to hold true both for plants grown under carefully controlled conditions and also for plants growing in the woods (28, 32, 49, 80, 99, 131, 149). Without exposure to full sunlight for at least a part of the day, plants do not develop satisfactorily (36). Light shade is sometimes beneficial, especially if the plants are grown under conditions of suboptimal water supply, since transpiration loss tends to be directly correlated with radiation intensity (103 a, 10).

Plants with adequate food reserves, if kept in darkness, have exceptionally long internodes, are devoid of chlorophyll, have only rudimentary leaves and poorly developed roots (150). If plants are exposed to light of very low intensity, the leaves unfold and become green and the internodes are somewhat



shorter. As the light intensity is increased up to a value of approximately 20 to 50 percent, leaves attain a maximum size. At this point the height of the plant is also maximum. A further increase in light up to full sunlight results in plants with a slight decrease in height, in length of internode, and in area of leaves, but with an increase in percentage of dry weight, in number of branches, in size of roots, and frequently also in flowers and fruit (1, 53, 63, 64, 28, 116, 117, 131, 146, 161, 177).

The actual light intensity required for the survival of plants, if other conditions are favorable, is low—one to five percent (52, 146)—and is close to the value at which photosynthesis balances respiration (23, 72, 158, 159). However, under field conditions, it is not possible for plants to survive indefinitely with such low intensities, since they must be able to produce sufficient food reserves to enable them to meet the other unfavorable conditions which almost always prevail. Plants can be found in hardwood stands where the intensity of light is below five percent, but Daxer (32) has noticed that with many of these plants, photosynthesis exceeds respiration only in the spring before the leaves of the overstory are developed. The real shade plant then is not so much one which can grow under conditions of low light intensity, but rather one which can exist for a long period under conditions which are unfavorable for photosynthesis. When exposed only to light of low intensities, such plants probably have a much lower basal metabolism than sun plants.

Light intensity also influences, to some extent, the mineral nutrition of plants, particularly their nitrogen metabolism. Unless plants receive enough light for rapid photosynthesis, they accumulate but cannot utilize nitrogen effectively and, conversely, if they do not receive sufficient nitrogen they accumulate but cannot utilize carbohydrates effectively (68, 108, 125, 126, 127, 128, 129, 130, 160, 161, 166). Apparently light intensity affects nitrate metabolism only indirectly through its influence on carbohydrates. It has been shown that corn plants will absorb nitrogen equally well whether the nitrogen is available only during the day or only during the night, and that both series of plants will attain approximately the same total dry weight (50 a). Light influences, but to less extent, the iron, calcium and potassium metabolism of plants (60, 96).

*Light Requirements as to Quality*

Recent studies on the effect of different wave-lengths of light on the opening of stomata have shown that they respond about equally to light in the yellow, blue and green regions, but respond somewhat more weakly in the red. Stomata ordinarily do not open in infrared (113, 134, 156).

Even though the light in forests is changed considerably in spectral energy distribution, it still contains a sufficient percentage of the various wave lengths in order to induce approximately normal growth of plants. While the red and blue have been found by many investigators to be somewhat more efficient in photosynthesis than the green, their findings are based on intensities of incident energy rather than intensities of energy actually absorbed by the leaves. Where care has been taken to make sure that equal amounts of energy are absorbed, the green region has been found to be not only valuable for photosynthesis (25) but to lie between red and blue in its relative efficiency (45, 179). Furthermore, some 40 to 70 percent of incident green is absorbed by leaves (138, 139, 140, 142) and this certainly cannot be dismissed as valueless (142) as some investigators have seemed to think (32).

When plants are deprived of blue, but grown with full complement of red and infrared, they develop many of the characteristics of etiolated plants (44, 51, 61, 77, 118, 120, 146, 162, 163, 167). This has led several investigators to conclude that red acts on the plants like darkness, and that blue has the same effect on plants as reduced "white light."<sup>6</sup> Recent studies by Johnson (74) and Arthur (6) indicate that the real factor causing the excessive elongation is infrared rather than red, and that this etiolated effect can be greatly reduced by increasing the intensity of light in the red region. Since sunlight is far richer in radiation within the visible region than the ordinary artificial lights used, plants grown even under reduced intensities of sunlight rarely show the pronounced etiolated effects characteristic of those grown with only red and infrared. Small changes in

<sup>6</sup> The term "white light" is used by many investigators to designate sunlight, or even artificial light in which all wave lengths are represented. When so used it covers a multitude of variations and is in no case adapted to precise expression. In the writer's opinion its use should be discouraged and the name of the actual source designated.

the quality of light, such as occur in incandescent lamps of different wattage and efficiency, produce no noticeable effect on the growth and structure of the plant provided the total intensity as measured in foot candles is the same (7).

The actual effect of blue acting alone on the growth of plants is not well known, since it is difficult to get blue of sufficiently high intensity to produce a rapid rate of growth. While it is claimed that blue acts mainly as reduced "white lights," *i.e.*, it produces plants normal in shape but smaller in size, the writer is of the opinion that blue has a tendency to cause shortened internodes and to produce a smaller, more compact plant than would be produced under full daylight of the same intensity (146). All of the studies made seem to point toward the conclusion that full sunlight as normally received at the surface of the earth, even though it varies greatly in quality, is far more satisfactory for the growth of plants than any portion thereof, or than any artificial source which has yet been discovered (104, 105, 106, 146, 168).

While reduction in the red and blue in the spectrum which occurs in the forest may slightly reduce the efficiency of the light (168), plants do live and survive there and the intensities required are not much greater than are required when normal daylight is supplied to them, but reduced in intensity by the use of neutral screens. Certainly plants grown in the shade of forest trees have not revealed any such pronounced changes in structure as are found in those plants which have grown under definite spectral regions (32). Therefore, it may safely be concluded that the changes in spectral energy distribution of light in the forest have only a secondary influence on the growth of plants on the forest floor, and are of far less importance than the change in intensity.

Some seven or eight years ago, rather elaborate claims were made as to the importance of ultraviolet radiation for plants. Experiments seemed to indicate that ultraviolet stimulated the rate of growth, that it was necessary for the prevention of etiolation effects, that it caused an increase in the yield of glucosides in *Digitalis*, and that it had a number of other vital effects (42, 100, 101, 102, 103, 144, 145). A critical review of most of this work has shown that other factors such as increased total light

intensity, increased intensity in the blue region, increased temperature, exposure to ozone, and many other factors which accompanied exposure to ultraviolet radiation may be responsible for most of the differences noted (91, 104, 105, 106, 121). Ultraviolet does have a pronounced injurious effect on plants, especially ultraviolet of shorter wave lengths than those found in sunlight (43). With prolonged exposures to high intensities of ultraviolet at 290 m $\mu$ , which is the short wave length limit of sunlight, slight injuries have been observed (9). Ultraviolet beyond that found in sunlight also renders the mechanism of photosynthesis inactive without any detectable change in chlorophyll (5).

A possible beneficial effect of ultraviolet on plants is its effect in increasing the vitamin D content (97) and also in favoring assimilation of calcium. Stewart and Arthur (164) have found that plants grown under conditions of low light intensity, if given exposures to ultraviolet, had an increased calcium and phosphorus content. No increase in these elements could be obtained in plants cultivated under full sunlight in fair weather. Similar effects of ultraviolet on calcium metabolism have been reported by Benedict (17). Ultraviolet light, or at least blue and ultraviolet, also appear to cause an increase in the assimilation of nitrate (170, 171).

The true role of ultraviolet in the region of 290–310 m $\mu$  in the growth of plants is still an open question, and while this region may have some favorable effects (182) its importance in determining the distribution of plants, as postulated by Ivanoff (71), is as yet a matter only for conjecture. Certainly, there is little justification for assigning to it the same importance as to intensity and duration of light.

#### *Other Factors Varying with Light in the Forest*

For a proper evaluation of the effect of light on the growth of plants in natural forest habitats, an understanding of the many other factors influenced by the forest is necessary. Wind velocity is reduced. Air temperature is usually reduced, but may sometimes be higher in small openings, particularly when cool winds are blowing; evaporation is decreased; and a number of other factors changed (2, 55). One factor which has until recently

received comparatively little attention, is the increased carbon dioxide concentration of the air in forests (32, 40, 98, 136). Just how valuable this factor is to the plant is not definitely known, but from measurements under laboratory conditions, it would seem that it may have a very important effect, especially when other conditions are also favorable for photosynthesis. Whether the roots of plants can actually absorb carbon dioxide from the soil which may be used in the leaves, is still open to question (95). In order for the carbon dioxide of the soil to be injurious to the roots of plants, it would have to reach a concentration of two percent by volume of soil atmosphere (40).

The factor which has received by far the greatest amount of attention besides light, is that of root competition. This was first called to attention by Fricke in 1904 (41) and has been emphasized particularly by Toumey (172) in this country. The roots of forest trees draw heavily upon the soil for both moisture and nutrients. When the roots of older trees are excluded from a small plat of ground by trenching around it, the plants within this plat usually show an important increase in their rate of growth and many new individuals may become established (15, 37, 38, 39, 30, 81, 173). This has led some individuals to ascribe to root competition the dominant role in determining the composition and growth of the understory (172). However, it has been pointed out that the root competition which tree seedlings must withstand is often just as great or greater in grassland (114, 115) and that during periods of severe drought, the ameliorating effects of the shade provided by the overstory enables plants to survive better in the forest than in the open (151). Studies of Fabricius (37, 38, 39) and others (30, 148) indicate that both light intensity and root competition are important factors in the growth of plants under forest canopies, and that improved growth can be brought about either by an increase in light intensity or by the elimination of root competition, or by both. Neither factor can safely be neglected.

Plants grown under light conditions which favor the accumulation of carbohydrate reserves are much more able to resist drought and frost, provided exposure conditions are the same, than plants grown with a minimum supply of light (33, 34, 152, 176).

Just what ecological effect length of day may have on the growth of plants under natural conditions, aside from its influence on flowering and fruiting, is not well known. It has been shown that only very low intensities of light supplementary to daylight are required to bring forth the photoperiodic response in plants (123, 124, 181). Undoubtedly, length of day through its influence on flowering, fruiting and dormancy, does have an important effect on the distribution of plants (46, 47, 48), but whether important effects on mineral nutrition are caused by length of day under natural conditions, is problematical.

### *Light and Succession*

Ecologists have ascribed to light a dominant role in plant succession. It is commonly assumed that the chief reason why a given species of forest tree fails to succeed itself is due to the inability of its seedlings to grow in the shade of the parent trees, whereas seedlings of the succeeding species are able to do so. From observations on plants grown under different light conditions both in artificial shades and in the forest, the writer has come to the conclusion that this conception is not entirely in accord with the facts and the following explanation is offered as being more probable.

When light intensity is too low for the growth (increase in dry weight) of species intolerant of shade, it is also too low for appreciable growth of the most tolerant. The real difference between the tolerant and intolerant species, in so far as light requirements are concerned, is the length of time they can survive in light intensities too low for growth. When light intensities are high enough for appreciable growth of either species, the one having the most rapid rate of growth, which is usually the least tolerant, will outgrow the other.

There is considerable difference in requirements of different species for germination and establishment of their seedlings. Seedlings of tolerant species are generally better able to become established on the forest floor; in fact, shade appears essential in some species (153). Once established they can exist for a long time without making appreciable growth, whereas the intolerant seedlings, if established, soon die out. This results in an understory made up almost entirely of tolerant species. When



favorable light conditions occur, due to the death or removal of a large tree in the overstory, the tolerant seedlings increase in growth and appropriate the openings before intolerant ones seed in.

Studies of plant distribution and growth in relation to light intensity have been made by a number of investigators whose findings are in accord with the explanation of succession given (3, 12, 65, 133, 148).

#### *Light Measurement<sup>7</sup>*

Light may be measured by measuring its chemical, illuminating, electrical and heating effects. All instruments based on the first three effects are selective in nature, that is, they are unequally sensitive to different wave lengths. Only methods which depend upon the heating effect of light are uniformly sensitive throughout the spectrum.

There has recently been a tendency on the part of plant workers to abandon instruments based on chemical and illuminating effects in favor of instruments based on electrical effects of light. Several photoelectric cells are available which may be satisfactorily employed for measuring light in the field. Recently a new cell has been introduced in Germany and in this country which does not require an external source of electrical potential for its operation. These cells are very convenient and are sufficiently rugged for field use. They are being adopted by a number of plant workers (7, 142, 178). These cells seem to be subject to many of the same shortcomings of conventional photoelectric cells. If exposed to the intensity of full sunlight, they are subject to solarization, which results in a decrease in the current produced with increasing exposure time (6), and they are, of course, selective in their sensitivity. The sensitivity curve of these cells is very close to that of the human eye, and for this reason their use is favored by a number of plant workers. However, it should be borne in mind that there is no evidence to indicate that the plant responds to the different wave lengths of light in the same degree to which the human eye does, even though photosynthesis is confined mainly to the visible region.

<sup>7</sup> A rather complete survey of methods of measuring light was published in 1931 (147) which deals with the advantages and limitations of the various types of instruments. Discussion here will be limited mainly to instruments and methods which have appeared since then, together with a discussion of the technique of taking measurements in the field.



A number of workers have been unwilling to use non-selective radiometers for measuring the light available to plants because of their sensitivity to infrared, which was supposed to exert only a heating effect on plants. However, with the recent discovery of the influence of infrared in producing elongation, this portion of the spectrum can no longer be neglected. The writer is of the opinion that non-selective radiometers may safely be used for measuring the intensities of solar radiation available for plants, and also for measuring artificial radiation, provided the spectral energy distribution is known. For measuring radiation in the visible region only, thermopiles and other non-selective radiometers may be equipped with either glass or solution filters which absorb infrared. Photoelectric cells, because of their high sensitivity, are more satisfactory for measuring narrow spectral bands when the energy output is low. Discussion of some of the newer photoelectric cells are given by Atkins and Poole (13, 14), Neurnbergk (112), Volk (178), Thomson (169) and Howlett (69).

Arcichovsky (4) has developed a new method of recording the intensity of sunlight by exposing photographic paper. New thermopiles have been developed by Withrow (180) and Brackett (22). Hall (54) described the use of thermometers having blackened and whitened bulbs, which may be used for rough approximations. Almost any instrument gives better results than a guess, since the human eye has such a wide range of adaptability to light that it is almost impossible to estimate light intensity accurately, even by practiced observers.

There has been some contention among ecologists as to whether light should be measured by using a plane or spherical absorbing surface, and if a plane surface is used should it be oriented normal to the sun's rays or exposed in a horizontal plane (94, 24, 49). It is argued that an individual plant does not have all its leaves in a horizontal plane, hence a spherical surface is preferable. This argument is sound when the investigator is concerned only with individual plants, but when the growth of vegetation on a given area is considered, then only the horizontal component of sunlight is available. Since weather observers usually base their measurements on the energy absorbed on a horizontal surface (76) it is desirable for plant workers to follow their lead, especially when comparing light conditions and growth in two widely separated regions.

Another point which merits consideration is the accuracy of measurements in the forest. Gast (49) has recommended a minimum of 40 complete daily records, well distributed throughout the growing season, to get a significant average of the radiation available beneath a canopy. Even then the value applies only to the particular point where the instrument is located. The writer has found that 20 to 40 direct readings made at uniform intervals over the area of a 1/10 acre plot will give an average accurate to within five percent if the canopy is not too uneven. Two averages determined in this manner on the same plot, but at different times of year, usually agree to within five to ten percent, especially if the measurements are taken with the sun at approximately the same solar zenith distance.

Most ecologists will be obliged to work with direct reading rather than recording instruments and each will have to determine for himself how many separate readings are required to obtain the desired degree of accuracy.

The development of instruments and technique for measuring light suitable for ecological use has advanced rapidly during the past ten years and as more and more ecologists adopt the newer methods, we may expect an increase of important discoveries in the field of light and its influence on vegetation.

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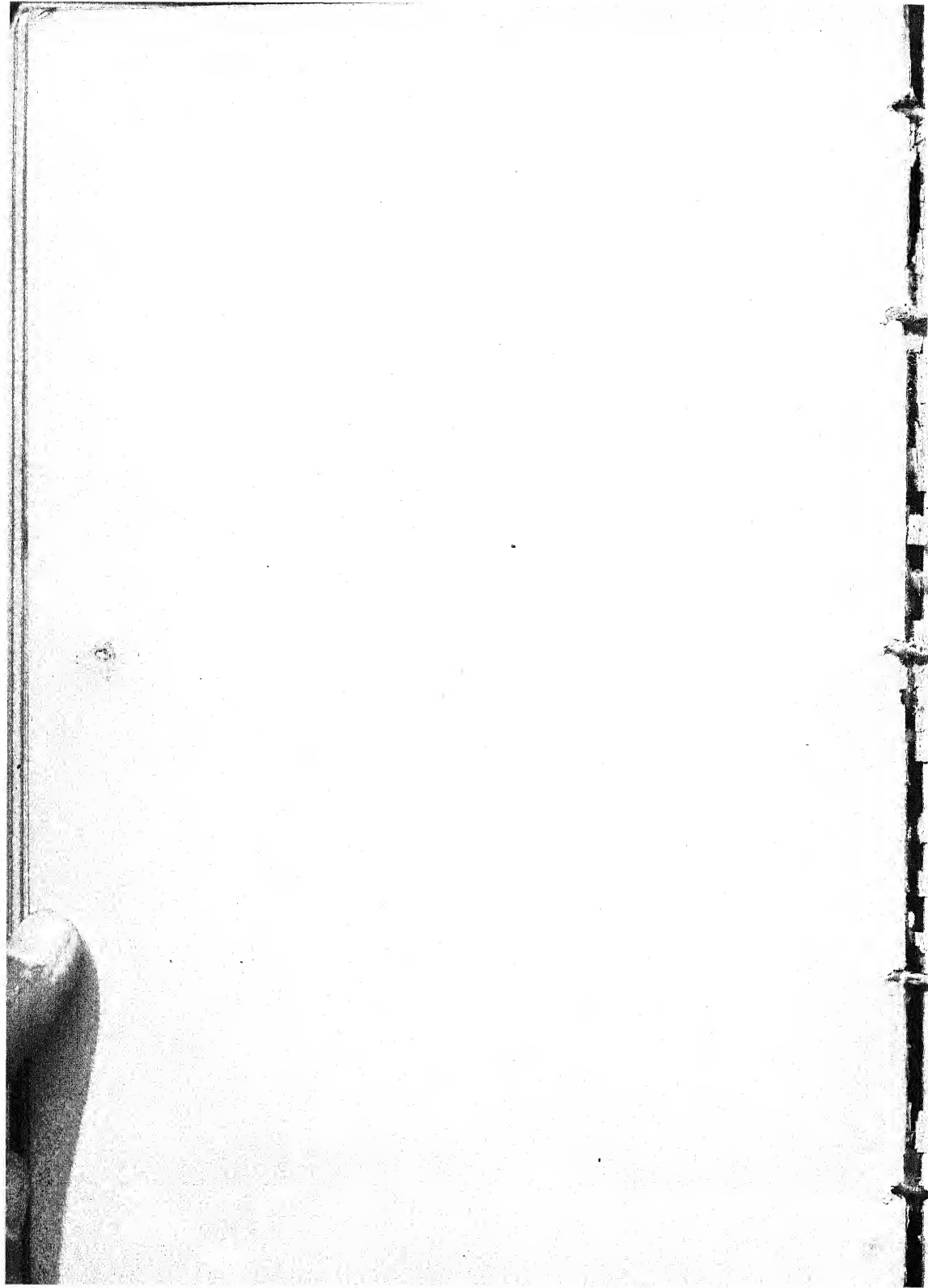
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# THE BOTANICAL REVIEW

VOL. I

OCTOBER, 1935

No. 10

## SOME VIEWS ON THE MORPHOLOGY AND PHY- LOGENY OF THE LEAFY VASCULAR SPOROPHYTE

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### AT THE PARTING OF THE WAYS

Dr. Scott, in the last edition of his "Studies in Fossil Botany," remarked that recognition of the Psilophytales had led us to look at the whole question of relationships among the higher classes of plants from a new point of view (53, 387). In the twelve years that have passed since these words were written, a number of fresh and curious types, either belonging to or suggesting affinity with the Psilophytales, have been described. While the problem of the interrelationships of the Pteridophyta has been perhaps complicated rather than solved by these discoveries, the importance for phylogeny of the Psilophytales, the predominantly Devonian phylum, has certainly been increased.

Is this phylum to be regarded as a primitive group of vascular cryptogams? Nearly all botanists seem to hold that even if the Psilophytales known to us are not the direct ancestors of geologically less ancient phyla, the group as a whole and, in particular, its best known family, the Rhyniaceae,<sup>1</sup> are of highly primitive construction and thereby give us the clue to the other pteridophytic phyla (27, 775; 28, 617; 30, 842; 5, 5-6; 7, 122; 54; 53, 420; 55, 155; 57; 22, 176, 692; 63, 108; 10, 494-501; 33). Church, however, does not regard the Rhyniaceae as Pteridophyta but as primitive Bryophyta in process of reduction from more freely branched algal forms. The theory of the origin of the Bryophyta by reduction does not concern us here, but the recognition that the plant-body of the Rhyniaceae, as known to us, is a vascular sporophyte,

<sup>1</sup> In the present article, this family is held to include *Rhynia*, *Hornea* and *Asteroxylon*.



such as is found among cryptogams only in the Pteridophyta (27, 779) is of fundamental importance. Even if it be not possible, as Church declares, to prove lignification of the tracheid-like cells in *Hornea*, owing to chemical changes during fossilization, it would still hardly be possible to interpret them, as Church does, as of the nature of the ringed unlignified cells found in some recent Bryophyta, in the face of certain sections (28, *pl. VIII*, *f. 47* and *pl. VIII*, *f. 48*). And *Hornea* does not stand alone; its anatomical similarity to *Rhynia major* makes it clear that both plants are constructed on the same anatomical plan. *Rhynia major* has a relatively well developed and preserved vascular cylinder. Church believes that the land was colonized by maritime algae in a single transmigration by the raising of the floor of the ocean, the rocks thus raised carrying with them all the plants growing on them, but that only the most highly organized of them—more highly organized than any *single* algal form at present known to exist in the sea—became the ancestors of the Pteridophyta. He holds that it "admits of no reasonable doubt" that these algal ancestors of the Pteridophyta were fine plants, three to six feet long. He also asserts that on leaving the sea they already possessed morphologically differentiated main axes, lateral and dichotomous ramification, root ("crampon") ramuli, definitely delimited "bilateral" ramuli (*i. e.*, leaves), Fibonacci symmetry, and "even axillary habit in the laterals" (11, 88-90). Later he lays down the general law that the initial stages of leaf-production are solely a phenomenon of the sea (12, 134).

It may well be that the primitiveness of the Rhyniaceae has sometimes been overstated. Kräusel, for instance, argues that in the Psilophytales we have true vascular plants, but of an organization so simple that no simpler one could be imagined (33, 7-8). Scott has suggested, on the other hand, that the Rhyniaceae, though primitive in general organization, may have undergone some reduction, as their peaty habitat suggests (53, 391). But that they are primitive seems to be shown by the structure of their sporangia. In *Hornea* the sporangium evidently arose by transformation of the tips of certain branches, and when one of these was in a condition of incipient branching the sporangium, with its columella, partook of this dichotomy. In *Rhynia Gwynne-Vaughani* Kidston and Lang found two unusual sporangia in which spores were

apparently formed at the end of a branch (28, 607); the word used is "stem," but branch seems a more suitable term) "without its being actually modified into a sporangium." The same writers draw attention to the fact that as early as 1908 Schenck had shown that the algal structures strictly corresponding to the pteridophytic tetrad of spores and the spore-mother-cells were respectively the algal tetraspores and tetrasporangia in which meiosis takes place (30, 851). Even if, as they suggest, we are here dealing with a homoplastic similarity which is an expression of general morphology rather than of relationship (28, 622), we can hardly fail to recognize in it an argument in favor of the primitiveness of the morphology of the Rhyniaceae and of their origin from a group with spore-producing organs of an algal type. Moreover, for the same reason and because of poorness of the geological record of the Anthocerotes, it is difficult to accept Campbell's view that the Rhyniaceae and the earliest vascular cryptogams were, if not actually Anthocerotes, at any rate probably much like them (9, 74; 10, 492). It may be noted here that Zimmermann's exposition of Campbell's views is misleading (63, 88); the views given as Campbell's are much nearer to, though not identical with, those of Church, and may have arisen by a confusion of these two conflicting points of view.

If the Rhyniaceae are reduced, there is every reason to believe that they are reduced from primitive leafless forms. Of recent years a number of leafless members of the Psilophytales have been described, as *Hicklingia* (31, 36) and *Taenioocrada*, under which Kräusel and Weyland include five species, the best known being *T. (Haliserites) Decheniana* Goepp. (37, 39). Only two will be shortly described here. *Gosslingia breconensis* Heard (20) was older, larger, and possessed a much better developed (exarch) protostele than *Rhynia* and bore its sporangia on a special short branch, variously interpreted and even compared to an axillary branch. *Pseudosporochmus Krejci* Pot. & Bernard was a large plant, reaching a height of over six feet.<sup>2</sup> It had a main axis up to 5 cm. in diameter, giving rise by repeated dichotomies to a crown of main branches. These themselves forked once or twice and gave off laterally a number of much shorter and more slender dichotomous branches, the ultimate branches bearing terminally

<sup>2</sup> The latest researches seem to show that estimates turning this plant into a small tree over nine feet high are based on remains that can not be safely identified as *Pseudosporochmus* (40).

narrow wedge-shaped sporangia which were themselves sometimes forked, presumably a primitive character. Neither of these plants can be regarded as reduced and yet they, too, are quite leafless. Zimmerman believes that not all the wedge-shaped expansions were sporangia and that some of them may be regarded as the first indications of laminar development (63, 111-112).

Moreover, Church's views involve, as Bower has pointed out, the tremendous assumption that in nature the colonization of the land by marine organisms occurred but once (6, 243). Bower also refers to Church's theory as one in which it is assumed that organic nature could not, or probably would not, originate new members in more than one phyletic line. Church, however, takes an extreme polyphyletic view of the origin of the land flora and regards not only the pteridophytic phyla but the flowering plants as lines that probably reached back independently to the sea (11).

It has come to be widely accepted that from dichotomous thalloid vascular sporophytes two main forms of leaf-bearing plants have been evolved, the small-leaved and the large-leaved. It is believed that primitively large leaves represent thalloid branch systems that have become flattened and appendicular with reference to a relatively main axis, but that primitively small leaves represent either emergences from a preëxisting axis or ultimate branchlets of a thallus. Goebel, however, saw no reason to doubt the homology of the leaf in ferns and lycopods, the typically megaphyllous and typically microphyllous groups. He held that the attempt to homologize the lycopod leaves with the paleaceous scales (he does not mention the *Aphlebiae* by name in this connection) of the frond and the frond itself with a flattened branch-system came to grief over the clear agreement between these scales and the hairs which in some ferns occupy their place. To look upon the hairs as reduced paleae would, he said, place too great a strain on a hypothetical construction already poorly equipped (15, 914). Those who support the emergence theory of the lycopod leaf would be more likely to derive the palea from the hair than *vice versa*. I have not been able to obtain a sight of the third edition of Goebel's book to see if he had changed his views.

#### THE EARLY MICROPHYLOUS FORMS

The Lycopodiales are typically microphyllous and a number of botanists believe that their leaves originated, phylogenetically speak-

ing, as emergences or outgrowths from a preëxisting axis. This view, formulated by Lignier (47, 48), has, in varying forms, received support from Halle (19, 36), Arber (2, 73), Hirmer (22, 691), Kräusel (33), Seward (61), Bower (7, 552), and other botanists.

More than twenty-five years ago Tansley pointed out that, while the possibility that the lycopod leaf owed its simplicity to reduction during phylogeny could not be excluded, yet, if the said leaf were held to be derived by foliar specialization of the short undivided branchlets of the thallus rather than of whole branch-systems, as in the ferns, it was possible "to bring the lycopods into line with the other Pteridophyta without either hypothecating extreme reduction or abandoning an explanation of the morphological nature of the leaf which seems by far the most rational and convincing that has ever been suggested" (62, 5, 9). And in 1921 Kidston and Lang were inclined to think that, on the whole, the organization of *Rhynia*, *Hornea* and *Asteroxylon* weighed against too sharp a distinction between large and small leaves and in support of Tansley's last mentioned view (30, 848). Lang, however, has since come to entertain the possibility that the simple leaves of some typical lycopods may prove to have arisen as emergences (44, 437).

Zimmermann accepts the origin of fern fronds from branch systems. His views on the origin of small leaves are difficult to summarize. He holds that in primitive vascular thalloid plants the plant-body was made up of units which he calls *telomes*. Each telome represents a single branchlet or segment, in so far as such are single and unbranched ("einachsrig"), according to the course of the vascular strand as well as in the form of the branchlet. Each telome starts, theoretically speaking, at its junction with another telome and ends (except, of course, in the case of terminal telomes) at the starting point of the next telome. Fertile telomes are represented by the sporangium, apparently with its vascular stalk (63, 65, f. 22); each sterile telome he calls a *phylloid*, using this word in a different sense from that of Lignier, who first used it for small leaves that are believed to have arisen as emergences. The telomes, fertile and sterile, are regarded as homologous and equivalent (63, 65).<sup>3</sup> To Zimmermann, large and small vegetative

<sup>3</sup> I can see no justification for Bower's statement that Zimmermann reduces the whole wealth of form characteristic of living Cormophyta to a fundamental organ, the telome, consisting of distal sporangium and a stalk, usually

leaves are combinations of sterile telomes, differing in the number of telomes that have, so to speak, been used up in the formation of the leaf. Though he speaks of each of his single "phylloids" as becoming directly converted by means of a greater or less amount of flattening and laminar development ("flächenförmige Verbreiterung") into a simple uninerved leaf, such as is found today in *Lycopodium* and *Equisetum*, this appears to be a description, in terms of telomes, of an ontogenetic process occurring today. In the same paragraph he adds "Often, e.g., in the Lycopsidea and Articulatae, there was at first in the position of a simple phylloid a complex of phylloids (Phylloidstand)" and urges that this indicates that the simple or "lyco-leaf" was in its earliest morphology equivalent to a freely branched branch-system of phylloids developing as a shoot (63, 65-67). On the following page he remarks that in its ontogeny and before branching sets in, every complex of telomes ("Telomstand") is a telome. And in discussing *Protolapidodendron Scharyanum* he says that this oldest of the Lycopsidea still shows us, as a vestige from its ancestors, the forking leaves, represented in all divisions of the Pteridophyta, but which do not occur later in the Lycopdiales (63, 130).

It is possible to look upon the hemispherical protuberances on the axis of *Rhynia Gwynne-Vaughani* as rudimentary emergences comparable to but more primitive than the spines of *Psilophyton*.<sup>4</sup> *Dutoitia pulchra* from the Devonian of South Africa might seem to support the idea of rudimentary emergences, since its dichotomous axes, terminating in relatively large and obconical organs, are highly reminiscent of *Rhynia* and some of them bear appendages "in the shape of hemispherical protuberances or spines which may attain a length of 1 mm." (23, 92, f. 1). Unfortunately, however, the vascular nature of *Dutoitia* is not established. Kidston and Lang, who first described the protuberances of *Rhynia Gwynne-Vaughani*, came to the conclusion that they did not represent an early step in the origin of small leaves, partly because they

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with vascular supply; nor for the further statement that in Zimmermann's view microphylls and megaphylls alike represent sterilized telomes (6, 617). The latter author regards fertile and sterile telomes (which, he points out, occur both separate and mixed in telome complexes, his "Telomstände") as originally homologous and equivalent. This seems a natural view for one who, like Bower (cf. 6, 490, 615-617), is inclined to look upon the vascular sporophyte as a generation homologous with the gametophyte (63, 86-88).

<sup>4</sup> Arber held that the two structures were equivalent (2, 24-25), but later researches have shown that they were markedly different.

originated late in the ontogeny and were frequently formed underneath a stoma. There seems, however, to be no particular reason why an emergence, ultimately serving a photosynthetic purpose, should not originate beneath or close to a stoma. Nor would the fact that these protuberances "cannot be regarded as part of the primary construction of the plant as at first developed" (30, 833) militate against the view that they are homologous with, or at any rate forerunners of, the spines of *Psilophyton* and the small leaf of *Lycopodium*; since these structures would, *ex hypothesi*, be phylogenetically new parts, arising from a preëxisting axis and not in the same morphological category as a primary part of the plant body. Lang's recent work on the spines of *Psilophyton princeps* has strengthened the view that these structures were morphologically emergences, probably of a glandular nature. Not only are they irregularly arranged and variable in size (from under 0.5 to over 2 mm. in length), but large and small spines are often inserted close together. Lang found no traces of vascular tissue in numerous and well preserved spines from Canada and Scotland (44, 43), so that there is a strong presumption that they were devoid of vascular tissue. Two fossils, probably belonging to the Psilophytales, seem to have had emergences even less leaf-like than the spines of *Ps. princeps*. In *Ps. wyomingense* Dorf from the early Devonian, the irregularly arranged spines were less dilated at the base, finer and closer together (14, 242). In this species the tips of the branches were not circinate, giving the plant a more *Lycopodium*-like appearance; the sporangia are unknown. In *Dawsonites Ellenae* from the Mid-Devonian of Norway both fertile and sterile axes bear a few spines which are usually very small, though they range from 0.5 to just over 1 mm. in length (25, 4, f. 1 and pl. II, f. 5). In this species the sporangia seem to be rather wider than long and are borne in clusters, much as in *Taeniochrada decheniana* (37, 38). On the other hand, vascular elements have been found in the spines of *Arthrostigma gracile*, a contemporary species so like *Ps. princeps* that only recently has it been shown that they were not parts of the same plant (21, 43). And in *Drepanophycus spinaeformis* from the Mid-Devonian rocks, another very similar form, the spine-like "leaves" show a midrib which was presumably vascular.<sup>5</sup>

<sup>5</sup> Kräusel and Weyland consider that this and *A. gracile* are certainly generically identical and may be conspecific, and on grounds of priority they would call the latter *Drepanophycus gracilis* (37).

From such appendages to the more leaf-like emergences of the Lower Devonian and Mid-Devonian genus *Asteroxylon* seems but a short step. For in this genus, besides the upper fertile naked branches of the *Hostimella*-type, there are regions of the axis which bear spines and are indistinguishable, when found detached, from *Psilophyton*. In this condition they are originally described as *Psilophyton princeps* (34, 182, pl. VIII, f. 14; pl. IX, f. 20). These spiny regions are in turn succeeded, toward the bases of the axes, by regions in which the spines pass gradually into densely set scale-leaves (35, 120). There is no evidence of vascular tissue in the spines or scale-leaves of either of the two known species of *Asteroxylon*, *A. elberfeldense* or *A. Mackiei*, though in the latter traces run from the axial cylinder to the base of the leaf (29). It seems significant that Seward should have established the genus *Haplostigma* for stems which appear, in the absence of petrified material, to be in some ways intermediate between Psilophytales from the Lower and Middle Devonian and more highly specialized arborescent lycopods from the Upper Devonian and Carboniferous. Of *Haplostigma irregulare* from the Devonian of South Africa, first described as a species of *Bothrodendron*, Seward remarks that in superficial characters it agrees more closely with *Cyclostigma*, a Devonian and Lower Carboniferous genus of lycopods, than with any other genus, but that it probably had a nearer relationship to the Psilophytales than to the Lycopodiales (60, 362). Some of the branches of *H. irregulare* were bare, having few or no scars or appendages, but some of the stouter branches bore oval or circular scars, spirally arranged, without any sign of a leaf-cushion, ligule, vascular bundle, or parichnos. There is some evidence that these scars mark the insertion of spiny emergences and that the very narrow cylinder was either fluted or irregularly stellate in transverse section, features which recall *Asteroxylon*. It is interesting to compare this form with *Cyclodendron Mathiewi* Sew. from what are probably Permian rocks of the Belgian Congo. Seward states that, while the systematic position of this fossil, of which no fertile parts are known, is not certain, there is a strong presumption in favor of including it among the Lycopodiales. The stems with their spirally disposed scars have a generally lepidodendroid appearance, though there are no leaf-cushions and apparently no ligules or parichnos. A single vascular strand seems to



have penetrated a little way into the very small scale-like leaves. Another feature strongly reminiscent of the Psilophytales is the very small size of the axial stele (1 mm.) and Seward notes that in its relatively small size as compared to the axis this strand agrees with the stele of a lepidodendroid branch. The proportions seem to be much the same as those found in some Psilophytales. It is not suggested that this late Palaeozoic fossil is a member of that ancient phylum, but merely that it has retained some primitive Psilophytalean characters and that it may show a phase in the vascularization of the emergence-leaf but little removed from that seen in *Asteroxylon*.

Thus, whether or not we regard the protuberances of *Rhynia Gwynne-Vaughani* as rudimentary prototypes of the spines of *Psilophyton*, we have a reasonably complete series of forms leading from the smooth axes of *Rhynia major* and other Psilophytales to forms with leaves like those of *Lycopodium*. It would seem natural to look upon the very small leaves of the Lower and Mid-Devonian *Protolpidodendron Scharyanum* and *P. wahnbachense* (38), in which both leaf and foliar bundle fork, as a further development of the emergence-leaf. As already mentioned, some botanists regard the leaves throughout the Lycopodiales as having originated during phylogeny as emergences. And it is certainly difficult to draw a line within the phylum dividing the leaves into two kinds. Among the Ligulatae, the leaves of some species of *Selaginella* are quite as small and, except for possessing a ligule, as simple as those of *Lycopodium*; and within the genus *Lepidodendron* are species in which the leaves are but a few centimeters long, while in others they reach nearly a meter in length, a size attained also by the leaves of some species of *Sigillaria* (22, 182, 251). In the latter genus, too, the foliar bundle was double, except in *S. Brardi* Brnt., where it was single but had two strands of protoxylem. But, given the large size of the axes of these plants, there is no reason for supposing that their leaves would not increase in size, even if originally emergences, or their foliar bundle fork. And *Sigillaria* is a geologically younger genus than *Bothrodendron* and *Lepidodendron* in which the bundle is single. Nevertheless, it would be rash to assert that all lycopod leaves have been derived from emergences, nor is it easy on such a theory to account for the ligule, its early

appearance and its constancy in certain groups. Is it an emergence from an emergence? Since we know that parallel development has been extraordinarily prevalent during evolution, it is by no means unlikely that, besides the series of emergences rising throughout the ages to the status of microphylls, there may have been in the Lycopodiales other series of forms in which simple, *i.e.*, unbranched, thalloid branches of a relatively high order became, as Tansley once suggested (62), converted into comparatively large and simple leaves. Such branches might well have possessed an emergence, retained during their evolution into leaves, which eventually became fixed in position and constituted the ligule. If this was so in *Selaginella* its leaves have undergone considerable reduction. Two doubtful but interesting fossils lend some support to this view. One, a nameless fossil of great antiquity from the Yeringian (Upper Silurian) rocks of Victoria (45)<sup>6</sup> seems to have had a stem usually over 1 cm. across and bearing long, narrow, simple, crowded leaves, about 2 cm. long and 1 cm. wide. In some cases they show a median line, probably representing a vascular bundle. Seward says of these remains that they differ "in appearance from any previously recorded plant" (61). They do, however, somewhat resemble a form from the Upper Devonian of Gilboa, New York, which Goldring described as ? *Sigillaria gilboensis*, while admitting that it was doubtfully a *Sigillaria* at all (17). The Upper Devonian plant is on a larger scale than the Yeringian one. Its leaves, when mature, sometimes exceeded 27 mm. in length, but in general proportions the plants are not unlike and the long simple leaves are a striking point of similarity. In neither case do we know anything of the fructification. Lang and Cookson, who described the Yeringian fossil, pertinently remark that, though it is natural to think first of the Lycopodiales, the probability must be borne in mind that some primitive plants had their lateral branches differentiated as long and simple leaves. If some of the larger adventitious branches, not infrequently found at the insertion of the hemispherical protuberances of *Rhynia Gwynnee-Vaughani* were to become flattened, we should get leaves of this type. The branch need not, of course, have been adventi-

<sup>6</sup> The age of these rocks, left doubtful in 1927, has since been confirmed (46).

tious, but such branches might well be unbranched and simple at origin and thus a step nearer to a simple leaf.<sup>7</sup>

If we widen our conception of the Lycopodiales and include *Barrandeina* and *Duisbergia* among them, or if we look upon these two genera as closely related to the primitive Lycopodiales, we should probably be inclined to take Zimmermann's view that the leaf of lycopods has undergone reduction. But there seems little ground for believing that these plants were lycopods. In *Barrandeina* (36, 40, 14) the decurrent bases of the leaves give the stem a superficially lepidodendroid appearance, but the leaves themselves were several times forked and showed parallel (or dichotomous) venation. In association with numerous German and one Norwegian specimen of *B. pinnata*, there were found fragments of a *Psymmophyllum* leaf. It seems likely, from the form of what remains of the leaves on the stem of both species of *Barrandeina*, *B. Dusliana* and *B. pinnata*, that some of the oldest leaves among those referred to the provisional genus *Psymmophyllum*, which clearly includes remains of more than one type of plant, belong to *Barrandeina* or allied plants. The identification of fertile parts of *B. Dusliana* supports the lycopod affinity, for it has been shown that numerous oval sporangia are associated with each leaf. Owing to poor preservation, the mode of insertion of the sporangia could not be determined. While they did not appear to be inserted directly in the axil of the leaf, it could not be ascertained if a "sporangophore" arising from the axis was present or if, as seemed to be the case in one or two places, a repeatedly forked structure was sterile distally and fertile proximally (40, 22). *Duisbergia*, too, hardly seems to be

<sup>7</sup> Since the above paragraph was written Lang and Cookson have published a fuller account, founded on further specimens, some of them of undoubted Mid-Silurian age, of the plant from the Yeringian rocks and have named it *Baragwanathia longifolia* (Phil. Trans. Roy. Soc. Series B. 224: 421. 1935). Some of the leaves of the new specimens reached a length of 4 cm., a size which does not materially diminish the gap in size between its leaves and those of the Gilboa fossil, since these latter sometimes exceeded 27 cm. *Baragwanathia* had a dichotomously branched axis and, in certain regions of the stem, reniform homosporous sporangia about 2 mm. in width and length were associated with and probably borne on the upper surface of spirally inserted leaves. The axial xylem was stellate in outline and without definite protoxylem or, so far as known, secondary elements. The xylem of axis and leaf consisted entirely of annular tracheids. The disposition of the sporangia strengthens the affinity with the Lycopods. As Lang and Cookson point out, *Baragwanathia* gives no support to the view that simple leaves were derived from emergences.

a lycopodial type, for the large and spirally inserted leaves with decurrent bases had numerous, apparently parallel but probably dichotomous veins. At the base of the lamina is a protuberance (a ligule?), while between some of the leaves are found cylindrical structures about 2-3 cm. long, believed to be sporangio-phores. In the best preserved specimens Kräusel and Weyland thought that towards the proximal ends of these they recognized two depressions, believed to be caused by two laterally inserted, swollen and more or less circular bodies. The axial stele consisted of very numerous, long, irregular bands of xylem, surrounded by a zone of radially disposed ("radial verlaufende") bands, associated in pairs (presumably leaf-traces). Kräusel and Weyland compare the stele to that of *Cladoxylon*, with which, as they point out, *Duisbergia* seems to have no real affinity, and to that of certain species of *Lycopodium* (36). Though the banded stele is reminiscent of similar steles of some species of *Lycopodium*, the leaves and double leaf-trace are very different. As we know of many cases of parallel stelar development, we can hardly regard the central cylinder with bands of xylem as an important indication of affinity. The presence of a ligule, if this should be established, would be a more definitely lycopodial feature, but, as Kräusel and Weyland are careful to point out, it is not proved that the protuberance of *Duisbergia* was of this nature. Moreover, *Duisbergia* shows no resemblance to any known ligulate lycopod. If the club-shaped bodies are sporangio-phores, the fructification is by no means lycopodial, unless Bassler's view that his *Gantheliophorus* was a sporangiophoric lepidophyte be accepted (3). This point lies outside the scope of the present essay and it can merely be pointed out that such a view is not generally accepted (4). Hirmer (22, 285) does not even mention *Gantheliophorus* and refers to it under the heading of literature dealing with *Mazocarpon*.

Kräusel in 1932 pointed out that it was doubtful if *Barrandeina* and *Duisbergia* represented a side branch of the Microphyllineae or a special group of the Macrophyllineae (33), and in a later paper he and Weyland leave it an open question whether *Duisbergia* should be associated with the lycopod or be placed in a separate group with *Barrandeina*. They even indicate, in passing, that if Nemejc's proposal to establish a new pseudomacrophyl-

lous group, the Noeggerathiales (50), should prove justified, *Barrandeina* might perhaps be brought into relation with *Noeggerathia*.<sup>8</sup> Until we know more of *Duisbergia* and *Barrandeina*, particularly of their fructifications, we can not, as Kräusel and Weyland truly remark, use them as a foundation for phylogenetic speculations, but on the present scanty evidence *Barrandeina* appears less remote from the Noeggerathiales than from the Lycopodiales.

#### THE FORERUNNERS OF MEGAPHYLLOUS PLANTS

Before the discovery of the *Rhynia* plants, Halle drew attention to the absence in the Lower and Middle Devonian of laminae even on well preserved fossils which showed most of the characteristics of fern fronds. He suggested, though with much caution, that the fern-like laminated frond of later geological horizons might be derived from a type such as that which he described as *Psilophyton Goldschmidtii*,<sup>9</sup> in which some of the lateral thaloid branches appear to have acquired slight dorsiventral symmetry and perhaps limited growth (19, 38). Seward, in commenting sympathetically on this suggestion, remarks that the development of a frond from bare lateral branch-systems is more clearly foreshadowed in the large, probably dendroid *Aneurophyton* (61, 122). This genus, *Cephalopteris* (formerly *Cephalotheca*) and *Protopteridium* (including *Milleria* Lang) appear to form a natural group.<sup>10</sup> In *Aneurophyton* the large main axis bore lateral and highly compound sterile branch-systems, the branching taking place in more than one plane. Except for the ultimate ramifications, which were very small and narrow expansions devoid of vascular tissue, there was no laminar development. The sporangia were borne on smaller separate branch-systems on which only an occasional sterile leaflet was present.<sup>11</sup> In the Upper

<sup>8</sup> For the affinities of the Noeggerathiales, see reference 8.

<sup>9</sup> As we are dealing with the mode of origin of the laminated frond and not discussing what genera gave rise to the earliest ferns having fronds, the fact that the remains united under *Ps. Goldschmidtii* are probably of doubtful specific value (44) or possibly belonged to various plants (26, 362) does not invalidate this suggestion. For the difference between such a phylogeny of characters (the "Merkmalphylogenie" of German authors) and ordinary phylogeny, see Zimmermann (63, 11) and Kräusel and Weyland (36, 352).

<sup>10</sup> *Eospermatopteris* Goldring (16, 17, 18) may have been a seed-bearing relative.

<sup>11</sup> For fuller accounts of *Aneurophyton* see Aderca (1) and Kräusel and Weyland (34, 35, 36).

Devonian *Cephalopteris mirabilis* Nath. the axis bearing the large branch-systems has not been recognized. Each secondary rachis at once gave off catadromically a short fertile branch which by rapid and repeated dichotomies gave rise to a mop-like cluster of very numerous sporangia. This first branching of the secondary rachis may probably be interpreted as an unequal dichotomy, the stronger shank forming the prolongation of the rachis. This view is supported by the fact that the next pinna borne on the latter was also catadromic, though thereafter the pinnae are alternately anadromic and catadromic. The secondary rachises are united in pairs, alternately on the back and front sides of the primary one, suggesting that the foliar bundle may have been of the tetrapolar type of the Dineuroideae (22, 516, f. 623, 624).

Of Protopteridium, four Mid-Devonian species have been recognized: *P. hostimense* Krejci, *P. Piedboeufi* Kräusel and Weyland (not yet described), and two species from Scotland, *P. (Ptilophyton) Thomsoni* Dawson (*Milleria Thomsoni* Lang) and *P. (Milleria) pinnatum* Lang. The Scotch fossils are represented by branch-systems, fertile in their upper part where a number of short lateral branches bear, probably after subdivision, terminal sporangia about 4 mm. long and under 0.5 mm. wide. The connection of these parts has been observed only in *P. Thomsoni*. The lower sterile branches are several times divided. In *P. pinnatum* the ultimate divisions are slightly flattened and widened, this being the only laminar development observed (41, 42).<sup>12</sup> *P. hostimense* consists of large and highly compound branch-systems, ramifications of the sixth order having been observed. Their construction is fundamentally sympodial and pinnate, but traces of dichotomous branchings remain (for further details see Kräusel and Weyland [40]). All the branches are cylindrical without any laminar expansion and contain a vascular strand. The elongate sporangia, 0.5–0.6 mm. wide and 3–4 mm. long, are borne terminally on filiform dichotomous branches. Kräusel and Weyland leave it an open question whether these branch-systems represent the main body of the plant or the branched rachises of a naked but otherwise frond-like appendage of an axis. In both *P. Thomsoni* and *P. pinnatum* the branchings seem to have been in one plane and this suggests that we are

<sup>12</sup> In *P. Thomsoni* the distal ends of the lower branches are not preserved.

dealing with a laterally borne dorsiventral member, that is, a compound frond, presumably borne on an axis (42).<sup>13</sup> Kräusel and Weyland say that the strand of *P. hostimense* at once reminds one of *Lyginopteris* and even more of the Lower Carboniferous stems (*sic*) of unknown attribution described as *Lyginorachis* (40, 8). The bundles which resemble those of *Protopteridium hostimense* are of course those of *Lyginorachis*, a genus founded for isolated petioles resembling those of *Lyginopteris*, and those of the petioles of *Lyginopteris*. The similarity, as they point out, is especially close with the vascular bundles of some recently described species of *Lyginorachis* (13, pl. II, f. 3) and this strengthens the view that the branch-systems of *P. hostimense* were appendages of an axis.

There seems to be some evidence that the Scotch species of *Protopteridium* were cryptogams and perhaps heterosporous. From the three localities in Scotland whence Lang got his specimens of *P. pinnatum* he obtained "multitudes of spores of a number of distinct types" (41, 255). Now seeds would be at least as likely to be preserved as these spores, but not a single example has been recorded. The evidence, though negative, is impressive from the number of spores preserved. As only about four other kinds of fossil plants have been recorded from these localities (41, 269), it seems a fair presumption that some of these spores belonged to *P. pinnatum*, though at the time, however, Lang was not able to draw even tentative conclusions about the groups of plants to which the eight different types of spores described by him belonged (41). Later he found an isolated spore attached to the wall of the sporangium of *P. Thomsoni* of the type previously described by him as type B (42). This is a somewhat specialized type of spore with a finely granulated wing; it is said to be common in the three localities from which *P. pinnatum* comes. There is thus at least a suggestion that this type of spore may belong to the latter plant. The spore is found in two sizes; in the larger the diameter is  $200\mu$  including the wing and  $175\mu$  excluding it, while in the smaller one, to which the spore in the sporangium of *P. Thomsoni* belonged, the corresponding measurements are  $140\text{--}150\mu$  and  $100\mu$  respectively.

<sup>13</sup> Kidston suggested that a large stem described as *Caulopteris Peachi* and found in the same locality as one of the specimens of *P. Thomsoni* might be the axis of this plant (42, 789).



The existence of two sizes in spores of similar type might as well indicate that the spores in question belonged to a heterosporous plant showing a definite but early stage of heterospory as that related species were concerned. That heterospory was in process of evolution in the Middle or Lower Devonian seems by no means unlikely. Lang notes that many of the spores from these formations are remarkably large for pteridophytic homosporous or pteridospermous microspores, though smaller than any of the megaspores known from the Upper Devonian or Carboniferous, or even than the megaspores of existing plants (44, 269). The largest type of spore described by Lang from the Cromarty beds in his type F, ranging from  $250\mu$  to over  $400\mu$  in diameter. It is said to be of general occurrence in all three localities but not usually abundant, a point which gives some slight support to the theory that it is a megaspore, since these are produced in smaller numbers than microspores or homosporous. It is presumably of this type that Lang thought when he wrote that only the very largest of the Cromarty spores approached the size characteristic of megaspores. In cases of incipient heterospory the difference in size between the spores would naturally not be great. In *Noeggerathia foliosa* megasporangia and microsporangia were externally indistinguishable (49) and this seems to have been true also of *Calamostachys Casheana* Will. (52, frontispiece; 22, 403, f. 487), where the megaspores were only  $180\mu$  and the microspores  $40\mu$  in diameter.

While it is generally admitted that the question is an open one, there seems to be a tendency to place *Aneurophyton*, at least provisionally, among probable pteridosperms. Kräusel and Weyland hold that there is no proof that this form was a pteridosperm, though there are some things in favor and nothing against such a view (35, 132). Seward places *Aneurophyton*, *Protopteridium* (as *Milleria*) and *Cephalopteris* among probable pteridosperms (58, 537; 61, 147, 153). Hirmer places *Cephalopteris* after the *Zygopterideae* and *Protopteridium* (as *Milleria*) among ferns, *sensu lato*, that are insufficiently known. The exclusion of *Aneurophyton* from the first volume of his textbook (the only volume yet published) seems to show that he looks upon this plant as probably a pteridosperm (22). The similarity between *Aneurophyton* and *Eospermatopteris* has undoubtedly contributed

to this tendency. The evidence is strongly in favor of *Eospermatopteris* being a seed-bearing plant, but, as Kräusel and Weyland remark, this is not definitely proved. As Zimmermann pertinently remarks, it might be a form showing marked heterospory (35, 132; 63, 251; 16; 17; 18). And, admitting that it was a seed-plant, that would not prove that all members of the group to which it belonged had evolved seeds.

Kräusel and Weyland's comparison of *Aneurophyton* to the Upper Devonian *Palaeopitys Milleri* and to the Lower Carboniferous *Stenomyelon*, especially to *S. tripartitum*, does not help us much as to its affinity. We know so little about *P. Milleri* that, as the last authors to describe it point out, it is not even certain if it was a pteridophyte or a gymnosperm (32, 416), though the xylem was of a typically gymnospermous type. Apart from the presence of a triarch or possibly exarch protostele in one very small specimen of *Stenomyelon tripartitum* (the larger specimens show three strands embedded in parenchyma), not a character necessarily indicating affinity, the chief similarities between the latter plant and *Aneurophyton* are the structure of its secondary wood and the absence of leaf-traces in the three fragments. These features, so far, are all that is known of it. But if, as has been suggested (53, 142), this latter character is due to the length of the internodes, then the apparent similarity is deceptive; while, if there really were no leaf-scars in *S. tripartitum*, then the species must be removed from *Stenomyelon*.

Admittedly, the anatomy of *Aneurophyton* and *Protopteridium* is more pteridosperm-like than filicinean.<sup>14</sup> It must be admitted that, as Scott remarked, the sporangia of *Protopteridium* are quite unlike anything known in the true ferns (56, 365). Such elongate and terminally borne sporangia seem to be more easily compared to those of the Rhyniaceae than to any other known sporangia. It is in the combination of advanced pteridosperm-like anatomy with a primitive form of sporangium and a relatively primitive morphology of the shoot that the interest of *Protopteridium* lies. May we not have in the latter a member of the group of thalloid vascular plants from which both the frond-bearing

<sup>14</sup> Well developed secondary wood was, however, characteristic of the Palaeozoic coenopteridacean *Botrychioxylon paradoxum* Scott (52, 319) and of *Zygopteris primaria* Cotta (57). Some secondary xylem occurs also in modern *Botrychium*.

groups, ferns and pteridosperms, took their origin? If it should be so, *Protopteridium* is a relatively advanced member of the group, not itself a common ancestor of ferns and pteridosperms. But in the form of the sporangia and in the absence of differentiation of the lateral branches, complex though they seem to have been in *P. hostimense* at least, as leaves, *Protopteridium* has retained characters, believed to be primitive, that have not yet been found associated in any fern or pteridosperm known to us.

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## EXPLANATORY NOTES

The geological periods mentioned, beginning with the most ancient, are the Silurian, Devonian, Carboniferous, and Permian. A land flora is generally considered to have first appeared in the Devonian. Even at the end of the Permian true flowering plants had not developed.

anadromous: having the lowest secondary branches originating on the anterior (upper) side of a pinna (Webster).

catadromous: opposed to anadromous, and having the lowest inferior segment of a pinna nearer the rachis than the lowest superior one (Webster).

columella: the axis of the capsule (sporangium) in bryophytes.

dichotomy: a system of branching produced by repeated forking of the growing point.

emergence: an outgrowth from the surface differing from hairs in arising from more than the superficial cells, and from spines in arising from a few layers only (Jackson).

exarch: having the protoxylem strands adjacent to the pericycle, toward the periphery of the stele.

Fibonacci symmetry: a symmetrical arrangement of parts in a system resembling the phyllotaxy of leaves.

heterospory: the possession of two different kinds of spores, distinguished by size or function or both, is characteristic of all seed-plants, of the water ferns, and the selaginellas.

homoplastic: having a similar structure or appearance not caused by phylogenetic origin from a common ancestor.

homospory: the possession of a single type of spore in the sporophytic generation, the homospore.

leaf-trace: the prolongation of the vascular system of the leaf into the stem.

ligule: a thin scale borne on the leaf above the sporangium in *Selaginella* and *Isoetes*. Plants with this structure are sometimes termed *Ligulatae*.

Lycopsidea: Vascular plants have sometimes been divided into two groups, the Lycopsidea and the Pteropsida. The former, abundant in palaeozoic time but represented today only by the Lycopodiales and Equisetales, are characterized by small leaves (microphylls), sporangia on the upper or adaxial surface of the leaves, and the absence of leaf-gaps. The Pteropsida include all other Pteridophyta and seed-plants and are characterized by large leaves (megaphylls), sporangia on the lower surface of the leaves, and leaf-gaps.

paleae: chaffy scales found on many ferns.

parichnos: two strands of tissue near the center of the leaf-scar in *Lepidodendron* and *Sigillaria*.

lepidodendroid: resembling *Lepidodendron*, extinct plants of the Lycopodiales, with dichotomously branched, often large stems and narrow crowded leaves.

protostele: a solid stele, characteristic of most roots and of many pteridophytes.

protoxylem: the first xylem or wood formed in the development of a vascular strand.

Psilophytales: a group considered to be the most primitive vascular plants and to connect the algae with the pteridophytes.

pteridosperms: ancient plants combining the habit of ferns with the possession of true seeds. Many supposed ferns of the Carboniferous period are probably pteridosperms.

Pteropsida: see Lycopsidea.

stele: the central cylinder of stems and roots, including the vascular tissues.

triarch: having three strands of protoxylem.

sympodial: branching by the development of lateral branches from a main axis.



## CONTEMPORARY UNDERSTANDING OF BACTERIAL PLANT-DISEASES AND THEIR CAUSAL ORGANISMS

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At the turn of the century, the study of bacterial plant-diseases had already attracted much attention and provoked considerable discussion. Today it has acquired an impetus and a significance which warrant its recognition as an independent branch of science.

After it had once been indisputably established that certain plant diseases are definitely caused by bacteria alone, it is not surprising that many diseases whose causes had previously remained unknown were also regarded as bacterial in origin. A most heterogeneous array of bacteria were then described as alleged causal organisms. Many of these earlier claims, however, have since been proved erroneous. At that time, for instance, it was said to be definitely established that *Bacillus amylobacter* is the cause of damp-rot of potato and that *Micrococcus populi* is the causal organism of a disease affecting poplars in southern France. Passalacqua (78) described a leaf-disease of aloe in Palermo, attributing it to a spore-forming bacterium to which he gave the name *Bacterium aloes*. There was indeed *a priori* evidence for these early claims, since spore-forming bacteria were already definitely known in human diseases, such as tetanus and anthrax. It is well to regard all these early allegations with skepticism, since not one of the spore-forming bacteria, among which *B. amylobacter* is numbered, is conclusively known to be a true plant parasite.

The cocci likewise play no rôle in plant pathology, in contrast with their importance in human and animal pathology. The alleged pathogenicity of *Micrococcus populi* has not been proved (83, 84) and the claims of Brussoff concerning *Micrococcus ulmi* as causing death of elm trees have already been refuted (104, 109, 126). This lack of evidence is significant, notwithstanding claims to the contrary, as those of Lendner (61), who attributes death of elms in the streets, gardens and parks of Geneva primarily to *Micrococcus ulmi* and *Pseudomonas lignicola* and only secondarily to the fungus *Ceratostomella ulmi*, which is the real cause.

There is one group of bacteria, however, which have acquired importance among plant diseases, although relatively unimportant in human pathology. These are the fluorescent bacteria, so named because of their ability to produce a green fluorescent coloring matter. Many of them are known to be pathogens and to cause serious damage to plants; for example, *Pseudomonas tabaci*, the cause of wild-fire in tobacco, and *P. medicaginis* var. *phaseolicola*, the cause of halo-spot of beans. The great damage caused by these fluorescent bacteria<sup>1</sup> is primarily a result of their ability to gain entrance into uninjured plant tissues, in contrast with other bacteria which can enter a plant only through wounds or similar injuries.

The manifestations of these diseases and the action of their causal organisms may be quite varied. While typical wilt-diseases, for instance, are usually produced through the partial stoppage of the conducting tissue by great numbers of bacteria which frequently more or less completely clog the vascular bundles, in other diseases there is definite destruction of tissues. In the latter, chlorotic discoloration of the green parts frequently develops, or fluids appear in the intercellular spaces and the diseased areas assume a greasy water-soaked appearance. Increased oxydase activity sometimes ensues from disturbance in the balance of oxydizing and reducing processes in otherwise healthy cells, manifesting itself in dark discolorations. Black-leg of potato is such an effect caused by *Bacillus phytophthorus*. In damp-rot, on the other hand, there is dissolution of the middle lamella and union between cells is thereby destroyed, normal turgor of the cells decreases, cell-sap exudes, and a more or less viscous decayed mass results.

Not only destruction of cells is caused by these pathogenic bacteria, but they may also cause abnormal cell division and cell size in the host plant, as is evidenced by crown-gall of fruit trees and other plants as the result of invasion of *Pseudomonas tumefaciens*. In other cases, hypertrophy may first develop with subsequent

<sup>1</sup> The generic name *Phytomonas*, while customarily used in America for fluorescent bacteria which cause plant diseases, is deliberately avoided here, since a distinction based solely on a character so inconstant as pathogenicity is not sufficient. See also Stapp, Schizomycetes, in Sorauer's Handbuch der Pflanzenkrankheiten, ed. 5, 2: 1-295. 1928, with mention of the older literature which, because of lack of space, can not be considered in detail here. For the same reason, authors' names are also omitted here after the names of host plants and species of bacteria.

destruction of the infected tissue. This is true of the pocket disease of sugar-beet caused by *Pseudomonas beticola*, of tuberculosis of olive caused by *P. savastanoi*, of canker, of oleander and ash caused by varieties of *P. savastanoi*, and of galls on *Gypsophila* caused by *P. gypsophyllae*. In this connection, mention may also be made of tumors on roots of *Coffea robusta* and *C. Klarinii* which Steyaert (116) attributes to *Bacterium coffeicola*.

Many species of the bacteria recognized as causing plant diseases are strictly limited to one host plant. Among these may be mentioned *Bacterium sepedonicum*, which can attack other plants after artificial inoculation but which in nature lives only on potato; *Pseudomonas stizolobii*, which grows only on *Stizolobium Deeringianum*, and *P. rubrilineans* which is confined to sugar-cane. Others are limited to a particular genus, as *P. translucens* on *Hordeum*, while still others can infect representatives of different and very remotely related families, such as *P. syringae*, *P. tumefaciens*, and *Bacillus amylovorus*.

It has usually been customary to bestow a new name on a disease and its bacterial cause if such a disease or any other with similar symptoms has not previously been known for the host concerned. In this manner many diseases and their causes have been enumerated during recent years, including the following:

Bacteriosis of pineapples (*Bacterium ananas*) in the Philippines (96).

Leaf-spot of *Ricinus* (*Pseudomonas ricini*) in Korea and Japan (47).

Spots on various organs of *Papaver Rhoeas* and *P. orientale* (*Bacterium papavericola*) (18).

On species of *Papaver*, including *P. somniferum* (*Bacillus papaveri*) (23).

Leaf-spot on species of *Viburnum* (*P. viburni*) (120).

Leaf-spot on *Berberis Thunbergii* (*P. berberidis*).

On *Carica papaya* (*B. papayae*) (82).

Damp-rot of sugar-beet (*B. betivorius*) in Korea (118).

Canker on plums (*P. mors-prunorum*) in England (127, 129).

Shoot-withering of plums (*P. prunicola*) (128).

On rice (*P. itvano*) (122).

On *Holcus Sorghum* and *H. halepensis* (*P. holcicola*) (30).

On *Phaseolus vulgaris* (*P. phaseoli* var. *fuscans*) (20).

- On *Cichorium Endivia* (*P. endiviae*) (56).
- On *Salix alba* (*P. saliciperda*) (68).
- On *Trifolium* and *Medicago* (*B. radiciperda*) (45).
- On *Iris* (*P. iridicola*) (119).
- On *Aleurites Fordii* (*P. aleuritidis*) (71).
- On *Eugenia latifolia* (*P. hypertrophicans*) (99).
- On pear (*P. utiformica*) (25).
- On tomato (*B. punctulans*) (17).

In connection with previously unknown diseases of a plant, consideration has frequently not been given to the fact that a newly discovered pathogen may be the cause of a disease in one or more other species of plants. In other cases, the same disease has been attributed to different factors in different countries. Such has been the case with black-rot and damp-rot of potato, which in Holland has been attributed to *Bacillus atrosepticus*, in Germany to *B. phytophthorus*, in Canada to *B. solanisaprus*, and in Ireland to *B. melanogenes*. A still more striking illustration is a leaf-spot of tobacco, which was found in North Carolina, described as "wild-fire," and attributed to *Pseudomonas tabaci*; another was described from Virginia as "angular leaf-spot" and connected with *P. angularata*; in Wisconsin the "Wisconsin leaf-spot" was associated with *P. mellea*, and finally in the Philippines an alleged fourth leaf-spot was attributed to *P. polycolor* (24). We shall later note that all four of these diseases are apparently identical.

Slight morphological, physiological, or cultural variations are not sufficient criteria for referring several diseases to different pathogens; neither can variations in the appearance of infected plants serve as the basis for such distinctions. Such variations indicate rather that every species of micro-organism possesses definite natural variability. It is known, for instance, that the same organism can form different colonies, some of rough, others of smooth appearance. Bryan (14, 15, 16) has observed in *Bacterium michiganense* not only the normal yellow but also a white or albino strain and a rose-colored race as well. Furthermore, she has likewise obtained white varieties of *Pseudomonas campestris*, *P. cucurbitae*, and *P. vesicatoria*, previously known only in yellow forms. Differences in virulence are associated with these variations in color.

There are, on the other hand, diseases which may be caused by different pathogens on one and the same host plant, such as damp-

rot on lettuce and endive. These damp-rots may be caused by any of the five bacteria *Pseudomonas viridilivida*, *P. vitians*, *P. marginalis*, *P. endiviae* (56), and *P. intybi* (112). These various bacteria are all distinctly different in physiological behavior and have only one character in common, that they all belong to the fluorescent group.

The number of species of bacteria described as entirely different from each other and causing plant diseases increases from year to year. Even the less critical plant pathologists may eventually wonder whether or not these innumerable species are legitimate. Fortunately, however, attempts have already been made to clarify the true status of these many species and such efforts may well deserve greater attention in the future. Comprehensive comparative studies of the species are necessary, involving serological tests in addition to the usual study of morphological, cultural and physiological characters. Not only agglutination reactions, but also precipitation reactions must be considered, since the former alone are insufficient while the latter are to be regarded as the most precise means of identification. When precipitation reactions are positive, identity of the species is unequivocally confirmed, even though there be definite cultural or physiological differences. If serological tests do not show complete identity, then we must group together those bacteria which closely resemble each other in producing similar pathological symptoms in the same host, and for the time being give them a single name, abandoning the others.<sup>2</sup>

Investigations of this nature have already been made on black-leg and damp-rot of potato, in which a total of 128 different races have been used, including the original cultures of *Bacillus phytophthorus*, *Bacillus melanogenes* and *Bacillus carotovorus*. Serologically these strains can be divided into five groups, the first one of which contains 111 of the 128 strains. In spite of the serological differences Stapp has proposed (101) the inclusion of all five divisions in one large group under the name *Bacillus phytophthorus*. He selected this name because the original strain of *B. phytophthorus* is one of the 111 races of the large first group, while *Bacillus carotovorus*, isolated and described from decaying carrots, matches a single other race, although its name has nomenclatural priority.

<sup>2</sup> Such a group is already known in medical bacteriology under the name *Bacillus coli* group.

Leach (59, 60), who has undertaken similar investigations, in part with the same pathogens, approves of this grouping but recognizes the priority of *B. carotovorus* and accordingly uses the name *B. carotovorus* group.

In a recent paper Stapp (107) describes a bacterium isolated from decayed hyacinths. It also belongs to the above-mentioned group, since it possesses features characteristic of the potato disease, but serologically it does not agree with the five sub-groups. This may be the same organism which Heinz first isolated in 1889 but described incompletely, to which he gave the name *Bacillus hyacinthi septicus*. Since this name is older than *B. carotovorus*, Leach must now refer to the group as the *B. hyacinthi septicus* group if he adheres to his principle of priority. That viewpoint would be justified if all the strains were completely identical, but since they are not, the name *B. phytophthorus* group may be retained, based on its most typical representative.

Link and Taliaferro (69) regarded the unlike agglutination tests of the two damp-rot bacteria, *Bacillus carotovorus* and *B. aroideae*, as evidence that these two otherwise closely related organisms are distinct species. On the other hand, recent results of Orton and Stanley (77) indicate whither serological investigations may lead if proper technique is not or only incompletely observed. They seriously maintain that numerous damp-rot bacteria, such as *B. aroideae*, *B. carotovorus* and *B. phytophthorus*, and even certain leaf-spot pathogens such as *Pseudomonas tabaci*, a fluorescent bacterium, should no longer be regarded as distinct species but as variants of the *Bacillus coli* group.

As Stapp (100) has shown and Patel (79) confirmed, various strains of *Pseudomonas tumefaciens*, the organism of crown-gall, are not serologically identical, although they are all included in the one *P. tumefaciens* group. Howard (40), on the contrary, found that strains of *Bacillus amylovorus*, the fire-blight organism, were serologically identical, even though secured from widely separated parts of North America and New Zealand. Williams and Glass (124) studied several strains of *Pseudomonas malvacearum*, the organism of the well known cotton bacteriosis and found them also serologically identical. According to Bryan (13), *Pseudomonas syringae*, the cause of lilac disease, is so similar to *P. citriputeale*, an organism causing black-pit of lemon, that they may be regarded

as identical. *Pseudomonas viridifaciens* and *P. vignae* may also be regarded as identical.

In 1930 Burkholder (19) made comparative studies of 18 species of *Phytomonas*; in 1932 Lacey (57) and in 1934 Clara (26) made similar studies of 15 and 19 species, respectively, of fluorescent bacteria. Additional comparative studies have been undertaken in recent years by others (36, 37, 75, 92, 98). All of these investigations have served to establish the identity of many so-called species of bacteria.

If Clara, who made comparative studies of two tobacco bacteria, had supplemented his physiological and infection experiments with serological tests, he would probably have been able to establish the identity of *Pseudomonas polycolor* and *P. angulata*. His suspicion that these bacteria represent one species was confirmed in 1930 when Stapp (103) showed that *P. angulata* and *P. tabaci* were serologically similar and consequently to be regarded as definitely identical.

The wild-fire bacterium of tobacco has attained significance in plant pathology because it is one of the few bacteria causing plant diseases in which toxin formation has been definitely established. This feature has long been known in organisms causing human diseases, such as diphtheria. As early as 1925 Johnson and Murwin called attention to the formation of toxin in *Pseudomonas tabaci*, but a detailed account was first given by Clayton (27) in 1934. According to him, *P. tabaci* forms toxin in all media in which it can develop. The toxin is thermostable, not precipitated by alcohol, neutral calcium acetate or lead acetate, and not destroyed by acids, but easily inactivated by dilute alkalies. When inoculated into leaves it destroys the chlorophyll, forming spots with chlorotic centers, typical of wild-fire. Such inoculations were successful on a whole series of plants, either with toxin alone or with toxin and bacteria together, and the reaction was stronger on many other plants than on tobacco. On these plants, in general, no infection was obtained by bacteria alone.

Development of technique for determining the resistance of tobacco to wild-fire is urgently needed, since the breeding of a resistant strain of tobacco is not only of scientific interest but of great economic importance as well. Stapp proposed a method in



1933 (108) which permits the inoculation of thousands of plants in a few hours.

A similar need obtains for determining the resistance of bush beans to halo-spot, *Pseudomonas medicaginis* var. *phaseolicola* (106). This disease, first discovered in North America in 1926, soon became very devastating and spread over whole continents. Numerous investigations have been made on the susceptibility of different kinds of bean (5, 21, 110, 111, 123, 130). There seems to be a few highly resistant varieties of bean in the trade and many which are very susceptible.

Jones (46) has published a method for determining resistance of alfalfa to the bacterial wilt caused by *Bacterium insidiosum*.

There is one bacterial disease, already mentioned several times, which for years has attracted unabated attention. This is the crown gall, found in all fruit-growing countries and caused by *Pseudomonas tumefaciens*. The patriarch of plant-bacteriology, Erwin F. Smith, devoted a good share of his life-work to the study of this disease and its cause. It is of much interest that this tumor, bearing many resemblances to those of animals and man, is caused by a definitely known bacterium, while a similar organism has occasionally been found in sarcoma and carcinoma but has not been considered as their cause. Though the causes of human cancer are admittedly various, they are not of a parasitic nature, even though a few irreconcilables have not yet forsaken the search for a causal organism. The alleged experimental proof that *Pseudomonas tumefaciens* is filterable, possessing a more or less invisible stage in its development, has also been discredited, together with the associated assumption of Lieske (66, 67) that this filterable phase might be responsible for human tumors (12, 49, 93, 95, 114). Similarly, definite proof of filterable phases of the bacilli of diphtheria, tuberculosis and typhoid has not been secured, in spite of numerous claims. Neither is it justified to draw general conclusions concerning the development and treatment of human tumors, as Lakhovsky has done (58), from apparently successful treatment of plant cankers. Lakhovsky claims to effect complete healing of tumor-bearing geraniums by binding them with coiled springs of copper wire of a particular diameter. Subsequent trials by Stapp and Bortels (115) have shown the complete futility of the claim.<sup>3</sup>

Schmidt (95) has also reported negative results in similar experiments.

Rivera (91) grew plants of geranium inoculated with *Pseudomonas tumefaciens* in chambers from which atmospheric rays, especially cosmic rays, were excluded to various degrees. In lead chambers cancerous growth developed rapidly and usually bore aerial roots, while in wooden compartments tumor-formation was slow. He concluded accordingly that certain rays which penetrated the wood, perhaps cathode rays, retarded cell division and conjectured that ionization of the air was also concerned.

According to Klein and his colleagues (51, 52, 53) the respiratory and metabolic activities of tumors are quite different from those of healthy tissues. In all plants there is a greater abundance of albumen in infected areas. The quantity of soluble inorganic and organic nitrogenous compounds as ammonia, amino-acids and amides differs in cancerous and healthy tissues, as well as the content of ash and acid. Catalase and peroxydase activities are also greater in the tissues of tumors.

Milovidov (72) was able to demonstrate an abundant development of typical plastids and of tannin in cancerous cells. On the basis of pH determination in tumors, Berridge (4) supposes that tumor-formation is associated with healing-reactions similar to those of wound-tissues.

Schimmel (94) has shown that the sugar content in hypertrophied tissues of the sugar-beet, tumors of which may become considerably heavier than the beets themselves, is generally less than in healthy parts of the same beet. Invert sugar, however, is always more abundant in the tumors. Since the ash-content may also be sometimes two or three times as great as in the normal tissues, Schimmel says there can be no doubt that the tumors draw food-stuffs from the beets.

Stapp and Bortels (113) have shown that development and form of tumors are largely dependent on the constitution of the host plant. Vegetative growth accelerates the formation of tumors, while flowering and fruiting exert an inhibiting effect. If vegetative growth of geranium, tomato or *Datura* is prolonged by early

<sup>3</sup> In a letter to the author dated March 5, 1934, Lakhovsky claims that slight inclination of the ends of the spiral toward the earth and orientation of its opening toward the north are necessary for success. Only negative results have been obtained in checking this procedure.

removal of developing flower buds, tumor development is favored; if the inception of the reproductive period is hastened, the contrary effect is secured.

There is still considerable discussion (62, 63, 76, 125) whether cancer-cells contain one or more nuclei or have an abnormal number of chromosomes. When Kostoff and Kendall (54, 55) inoculated tomatoes and later severed the stem above the tumor, sprouts occasionally developed from the tumors. If these sprouts, with part of the stem and tumor, were planted in earth, the roots which developed from them about 3 cm. above the tumor were all tetraploid; they possessed 48 chromosomes, while the original plants had only 24. In certain parts of the sugar-beet cells were found with 36 and even 72 chromosomes, while the normal number is only 18. Such tetraploidy is not regarded as the cause of tumor formation, although it may be an important factor in the development of new species. Tumors may develop upon the aerial parts of a plant as well as upon the subterranean organs. Barrett (1) has reported a genuine case of aerial crown-gall on the Golden Ophelia variety of greenhouse rose, among more than 3,000 plants of which not one was uninfected.

Recent investigations have also dealt with the biology of the crown-gall organism. Kauffman (50), Israilsky (42) and Israilsky and Starygin (43) have discovered two growth forms of *Pseudomonas tumefaciens*, the rough and the smooth, which frequently appear in cultures. Israilsky (42) claims to have isolated this organism or a very similar one from the tubercles of lupines; even the fixation of nitrogen is attributed to them. Such surprising discoveries must however be accepted with reserve.

Stapp and Bortels (114) have observed in living preparations the formation and disintegration of the so-called bacterial stars. These result from the assembling of separate motile rod-cells with protoplasmic connections. Star formation is distinctly favored by the addition of iron and manganese to the nutrient medium, by an abundant supply of oxygen, and by a high ratio of carbon to nitrogen in media with a relatively low supply of foodstuffs. After plasmic coalescence the disintegration of the star takes place and the protoplasm loses the viscous thread-producing nature which it had during the formation of the star. The individual cells again begin to divide and the young cells to swarm. ✓ The significance of

star formation is still undecided. There is at present no proof that it is a sexual process and the claim that it is such is very premature.

Rivera (90) claims that the crown-gall organism is injured by radium. He assumes that the gamma rays inhibit its growth while the beta rays cause its death.

It has long been known that *P. tumefaciens* can enter a host plant only through wounds and afterwards form tumors (80), but some supplementary experiments by Schmidt (95) may be mentioned. Schmidt germinated sterilized sunflower seeds on agar plates carrying a culture of the crown-gall organism. After 10 to 22 days the seedlings were transplanted into pots in soil; in no case did tumors appear on the plants. Fresh cultures of *P. tumefaciens* were smeared on pieces of rubber and tied to plants 10 to 25 cm. high with uninjured epidermis; the results were all negative. Plants were then placed in a partial vacuum and covered with an aqueous suspension of the bacteria. After re-admission of the air, the liquid penetrated the stomata and partially filled the intercellular spaces; on further growth of the plants the results were still negative, while in slightly injured plants tumors developed in every case after similar treatment. Banfield reports (2) the occasional presence of the bacteria in quantity on the surface of the tumors and has also found them in water in which tumors had been lying a short time.

There have also been recent investigations on the rate of transmission of *P. tumefaciens* within plant tissues. Hill (39) observed penetration in young tomato stems at the rate of 0.04 mm. per minute for the first quarter-hour and 0.03 mm. per minute for three hours thereafter. Since Ivanoff and Riker found the rate of diffusion of dead bacteria and of india ink to be the same, they conclude that the spread of the organism in tissues depends primarily on purely physical forces such as capillarity and negative pressure.

Bacteriophage has heretofore played no part in phytopathology. We may well give consideration to the interesting investigations of Chester (22), supplementing previous research on bacteriophage in the crown-gall (12, 73). He sought for bacteriophage both in and on the host plant and found it in healthy tissue adjoining the crown-gall in stems of geranium, but not in healthy uninfected plants. In the beet he found it in healthy uninfected plants.

From this he concludes that bacteriophage can diffuse from the point of infection into healthy tissues and that it can penetrate into healthy sugar beets directly from the soil, where it must be present. Chester also produced crown-galls by using strains of *P. tumefaciens* completely free from bacteriophage and thereby contradicted the claim of D'Herelle and Payre (28) that tumors are provoked by this organism only in connection with an invisible form. Gäumann (34) supposes that bacteriophage is not formed by destruction of the bacteria, but that its development is connected with a living reaction of the infected tissue. He concludes there must in some way be concealed here an immunizing reaction, a production of anti-bodies.

According to Thung (121) the crown-gall organism does not form toxin in bouillon cultures. He agrees with Magrou (70) in believing that its ability to form tumors may be attributed to the action of mitogenetic rays.

The number of plant families which are known to include hosts for the crown-gall has also increased. Muncie (74), for example, found crown-gall tumors on the roots of dock, *Rumex crispus*, in a raspberry field in Minnesota and on the rhizomes of rhubarb, *Rheum raponticum*, in a nursery in Iowa. Crown-gall has also been found on cypress, *Cupressus arizonica*, by Brown (6), on willow, *Salix caprea*, by Rainio (81), on cedar, *Juniperus Sabina*, by Brown and Evans (11), and supposedly on elm by Emery (32).

Levine (64, 65) succeeded in producing tumors on the giant cactus, *Carnegiea gigantea*, and on prickly pears, *Opuntia*, sp., by artificial inoculation. As early as 1926 Stapp successfully inoculated *Opuntia* (results not yet published), resulting in a tumor the size of a fist. This plant, now nine years old, has heretofore appeared to be alive, but since 1933 has lost all its cladodes above the tumor.

Since the first basic investigations of E. F. Smith and his colleagues on crown-gall in 1911, it has been assumed that the deformity of apple trees known as hairy-root is also caused by *Pseudomonas tumefaciens*. In 1929 and 1930 Riker and his colleagues gave proof that hairy root is caused by *P. rhizogenes*, a species closely related to *P. tumefaciens* but not identical. Inoculation with *P. tumefaciens* produced only crown-gall; with *P. rhizogenes* only hairy-root was induced, and with mixed infections

both effects were secured. The results were the same whether the plants were grown in steam-sterilized soil or in untreated soil (87). Inoculations were also successful with a series of other plants, such as rose, sugar-beet, bean, and chrysanthemum, but only weak infections, if any, were secured on tomato or tobacco. Suit (117) isolated the same organism from *Spiraea Van Houttei* and *Spiraea prunifolia*. In 1934 Hildebrand confirmed Riker's results on the causal differences between hairy-root and crown-gall. According to him, *P. rhizogenes* is a typical wound-bacterium; he found it in large quantities on the outer surface of hairy-root tumors and frequently in the earth surrounding them. Within the tissues it was localized in the intercellular spaces. Hildebrand found great differences in the susceptibility of apples to this disease. He tested twenty-nine kinds in 1930 and noted variations from 12 to 100 per cent, and the following year found variations from 22 to 100 per cent in thirty-seven kinds. Previous infections did not prevent subsequent attacks. Harris (35) also found differential resistance among apple stocks, demonstrated both by the relative number of successful inoculations and by the size of the tumors.

Riker and Hildebrand (88) have shown that the incubation periods of hairy-root and crown-gall are relatively long in spring and autumn and shorter in summer. Temperature and active growth of the trees are apparently important factors. Root-infesting insects in the soil, such as Phyllophaga, Elateridae and Mycetophilidae, may play an important part in the spread of the diseases. Riker and others (89) have shown that hairy-root is apparently more abundant than crown-gall in nurseries in the United States, while in Germany it occurs to a very limited extent, if at all.

As a preventive measure Riker has advocated binding the young trees with tape at the contact between stock and scion. This method is scarcely applicable in Germany, where a different method of propagation is used, obviating the danger of infection at this point. On the other hand, Oppenheim's method of immersion in "Uspulun-Lehmbrei" has been found satisfactory and has been further recommended by Husz (41) in Hungary and by Boumann (4) in Holland. It is still undetermined whether treatment of the soil with chlor-picrin, with which Kapshuk (48) has experimented in Russia, will be of practical value.

In conclusion there may be mentioned the interesting investigations of Eglits (29) on temperature and respiration in healthy and decaying potatoes. He found that the heat of healthy potatoes is about  $0.005^{\circ}\text{C}$ . above the surrounding temperature. If the potato was wounded, there ensued within an hour a rise of temperature in the wounded tissues to about fourteen times the normal amount. This wound-temperature fell at first sharply and then slowly with the healing of the wound and reached normal in about two days. If the tubers were inoculated with *Bacillus phytophthorus*, the temperature rose with some fluctuations for 170 hours to 30 to 35 times the normal. At this temperature the tissue died and in consequence the temperature fell rapidly to subnormal. The curve of respiration rose similarly, reached its maximum 30 to 40 hours before the death of the tissue, and then fell rapidly. Thus there is a characteristic curve of increased temperature and respiration in diseased plant tissue, just as in the diseases of man, but the "fever-condition" in plants decreases with increasing distance from the seat of infection.

This short review, covering only a portion of the recent literature, may serve as introduction to a specialized field of botany which is of both scientific and economic importance.

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# THE BOTANICAL REVIEW

VOL. I

NOVEMBER, 1935

No. 11

## SOME VIEWS ON THE MORPHOLOGY AND PHYLOGENY OF THE LEAFY VASCULAR SPOROPHYTE (CONTINUED)

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### THE MEGAPHYLLUS TYPES AS EXEMPLIFIED BY THE FERNS

We have already briefly considered (11a) the leafless predecessors of leafy vascular plants, the early microphyllous forms and the forerunners of the megaphyllous types. We must now deal with the typically megaphyllous phylum, the Filicales, *sensu latu*. So far as our present knowledge extends, the Coenopteridaceae are, phylogenetically speaking, the most important group of primitive Filicales. Professor Bower's latest book contains a diagram in which the multitudinous groups of recent ferns, eusporangiate as well as leptosporangiate, are shown as diverging from the Coenopteridaceae (9, 463). This diagram is not meant to convey the idea, however, that existing families of ferns are descended from any coenopterids known to us, for the author says that the Coenopteridaceae "may be accepted as synthetic types, representing more than any others such approximate sources as those from which the class of the Filicales may have originated" (9, 459). Scott's position was essentially similar, for he pointed out that while the Coenopteridaceae (his Botryopteridaceae) were too specialized to be the ancestors of modern ferns, the analogies that they present with so many fern families suggest that both groups represent offshoots from the same main line of descent (46, 364). And later he recognized the probability of "a real if somewhat remote affinity" between the Coenopteridaceae and Ophioglossaceae (47, 413).

Before considering the Zygopterideae and Botryopterideae, which with the less well known family of the Anachoropterideae constitute the order of the Coenopteridaceae, something must be

said of the scale-like structures known as *aphlebiae*; they are found chiefly in the Zygopterideae but also in some other Palaeozoic ferns. The *aphlebiae*, originally described as scale-leaves, were interpreted by Dr. Paul Bertrand as equivalent to secondary rachises, the emission of traces to which took place precociously before the leaf-trace had entered the petiole or secondary rachis (4). By far the most satisfactory analysis of the morphology of the *aphlebiae* yet given is by Dr. Hirmer (21, 573, 690). He distinguishes between true *aphlebiae* and *aphlebioid* structures. The latter may, phylogenetically speaking, represent modified pinnae, or structures of a stipular nature, or, perhaps even in some cases, complete specialized fronds, such as those found in the recent *Trichomanes aphlebioides* Christ. (51). It seems to be largely the failure to draw this distinction which has made certain writers despair of establishing the homology of the *aphlebiae* (22, 103). Thus, the so-called *aphlebiae* of *Diplolabis Römeri* Solms and *Metaclepsydropsis duplex* Will., found at the base of the insertion of the primary [not, as Dr. Holden suggests, at the base of the tertiary (22, 104)] pinnae have been shown by Dr. Gordon to be specialized pinnae (15, 716). Curiously enough, Dr. Hirmer alludes to these two species as possessing true *aphlebiae* (21, 690). And the so-called *aphlebiae* of *Austroclepsis (Clepsydropsis) australis* Osborne are, as Dr. Sahní pointed out in describing this fossil, fairly certainly the homologues of the primary pinnae borne farther up on the petiole (41, 15). While such *aphlebioid* structures may be large or small, the true *aphlebiae*, though they may be branched and possess a branching vascular strand, remain relatively small and represent, phylogenetically speaking, former microphyllous emergences, comparable to those of some Psilophytales, e.g., *Asteroxylon* and *Psilophyton*. Such emergences are held to have originated when the plant body was still thalloid and this would explain why in some species, e.g., *Ankyropteris corrugata* Will., *A. scandens* Stenzel, *A. Brongniarti* Ren.<sup>1</sup> and *Botrychioxylon paradoxum* Scott, they occurred both on the axis and on the

<sup>1</sup> This species is not mentioned in Dr. Holden's summary of the species of *Ankyropteris* possessing *aphlebiae*, but these were described by Renault as scale leaves and are shown in his figure (38). These figures also show that the petioles were not, as has been stated, whorled (21, 527), but were given off singly. Where their insertion is not disturbed by the presence of a petiole, it is the *aphlebiae* that might be described as being whorled or in a close spiral.

leaf. Dr. Hirmer also holds that the relegation of the (true) aphlebiae to special positions on the frond is a derivative condition, occurring in conjunction with a sharper definition of spatial relations. Dr. Scott, in a review of Dr. Hirmer's book, spoke favorably of the latter's account of the aphlebiae and aphlebioid structures (48, 344), and in his latest book Professor Bower expresses fundamentally similar views (9, 628). Scott had previously accepted the view that the aphlebiae were equivalent to secondary rachises and that they had in certain cases "spread" to the stem (45, 60).

In the case of the Zygoterideae the most controversial issue is whether the Dineuroideae, where the primary rachis bore four rows of pinnae, or the Clepsydroidae, where it bore but two such rows, was the more primitive. The decision on this point is much influenced by the view taken of *Stauropteris*. Lignier regarded this genus as much more primitive than the other coenopterids which he believed to have arisen from it or from forms allied to it. He even believed it possible that the branch-systems known as *Stauropteris* were not borne on a specialized stem (37). Dr. Hirmer also regards *Stauropteris* as very primitive and derives the other Zygoterideae from some such type, holding that the rachises of the second and, when present, of higher orders, came in the remaining Dineuroideae, presumably in correlation with the exigencies of available space, to be inserted laterally on the parent rachis and to share its plane of symmetry. He considers that in the Clepsydroidae the process was carried a step further and that the primary pinnae underwent the same change in orientation (21, 688).

Dr. Zimmermann emphasizes very strongly the morphological primitiveness of *Stauropteris* when he says (52, 188): "In other words, the whole structure still represents, as in the Rhyniaceae and without marked division of labour, the shoot-axis, 'leaves' and 'pinnae' . . . ." Its branch system is held to show an advance on the *Rhynia*-type by being built on a rigidly alternating system of monopodial and dichotomous branching, the pairs of pinnae consisting, properly speaking ("eigentlich"), of a completely reduced lateral branch which dichotomizes at once on leaving the parent axis (52, 189). This dichotomy does not seem, in Dr. Zimmermann's view, to represent a doubling in phylogeny, such as that

hypothesized by Drs. Bertrand and Sahni, but merely a primitive character retained and taking place precociously in the ontogeny of the frond. Dr. Zimmermann's view of the origin of the flattened and more or less typical frond from some such type as *Stauropteris* is, in essentials, similar to Dr. Hirmer's. Nevertheless, he holds that the petiolar bundle was primitively bipolar, differing only slightly from the *Clepsydropsis*-type in form and in the absence of the small islands of parenchyma abutting on the protoxylems. From such a prototype he derives three or more (52, 195) main series of zygopterid bundles. Of these, the stauropterid line arises by the antero-posterior division of the two lateral protoxylems and the lobing of the outline of the metaxylem to form the characteristic four-armed *Stauropteris* bundle, giving off vascular strands to four series of branches. In two other main lines the next step is held to have been the median constriction of the bundle and the appearance of two small islands of parenchyma abutting on the protoxylems. Such islands have also been observed once in *Stauropteris* (6). A bundle of the kind found in *Clepsydropsis antiqua* Unger thus results. Doubt has recently once more been thrown on the zygopterid affinity of this species (44), but the argument need not be affected thereby, as a clepsydroid bundle occurs as an early phase of leaf-trace development in several undoubted zygopterids. From this type Dr. Zimmermann postulates the evolution of a series culminating in the biseriate ankyropterids and of another series leading through the quadriseriate *Metaclepsydropsis* to the etapterids. Thus, for him, the similarities between *Stauropteris* and the petiole of *Diplolabis* are due to homoplasy. Dr. Bertrand, whose views on the evolution of the Zygopterideae are very different, also takes this view (5, 225). Professor Bower apparently shares in this opinion also since, while fully recognizing the doubt that subsists as to the morphology of *Stauropteris*, he speaks of it as the most archaic type among Palaeozoic ferns and as one giving a valid base for comparison with the Psilophytales (8, 269; 9, 459); he looks upon *Diplolabis* and the other quadri-seriate zygopterids as derived from biseriate types by dichotomy of the primary pinnae (7, 27). He regards the bipolar bundle as primitive for the family (7, 27; 9, 345). Dr. Leclercq, though she speaks of the suppression of the second or accessory plane of symmetry of the petiolar bundle of certain Zygopterideae leading

to the reduction of the rows of primary pinnae to two, yet seems to accept (in agreement, she says, with French and English authors), the *Clepsydropsis*-type of bundle as primitive for the petioles of the family. She rejects *Clepsydropsis* itself as the direct stock from which *Metaclepsydropsis* might have been evolved on account of its being a biseriate type. But she thinks it not improbable that a form with a clepsydroid foliar bundle, giving off pinna-traces to four rows of appendages, may one day be found to connect *Metaclepsydropsis* and its derivatives with the hypothetical ancestral bipolar bundle (35, B53, B58). Dr. Zimmermann argues that a plant such as *Stauropteris* may be primitive in certain characters, e.g., in external morphology and sporangial structure, yet divergently differentiated in other characters such as the disposition of the vascular elements. This is undoubtedly true and the fact that its sporangia seem to be primitive does not imply that the vegetative parts of *Stauropteris* are necessarily also primitive. But the form of the meristele and more especially the development of the lobes or arms of xylem, not only in *Stauropteris* but also in the petiolar bundles of *Diplolabis* and *Etapteris*, seem clearly to be correlated with the emission of four series of traces. So that if the flattened bipolar bundle was primitive for the Zygopterideae, then it would seem natural to regard the quadriseriate types as derived from the biseriate ones by a dichotomy, in the phylogeny, of the primary pinnae, and to look upon *Stauropteris* as a specialized zygopterid. This was the view taken by Kidston and Gwynne-Vaughan (25, 469), by Drs. Bertrand and Sahni (4, 5, 39) and provisionally by Scott (46, 337, 415). Drs. Gordon and Holden also incline to the view that the primitive zygopteridean petiolar bundle was an oval bipolar one and the latter author further thinks the biseriate forms with permanently closed filaments less specialized than the quadriseriate forms with their more modified secondary and tertiary traces and more strictly localized protoxylem (15, 731; 16, 187; 22, 107).

A new complexion has been put upon the problem of the relation of the quadriseriate to the biseriate zygopterids by Dr. Holden's researches on *Ankyropteris corrugata* Will. This species seems to be primitive in that in it the aphlebiae occur indiscriminately on leaf and axis, and in that it has no axillary branch, the stem branching by equal dichotomy. Dr. Holden has shown that it had

no flattened lamina or pinnae and that the occasional branching of the petiole was always dichotomous and, in the cases observed, at right angles to the bar of the petiolar bundle (22, 97). While this form of branching is almost certainly highly primitive, its occurrence in a genus included among the biseriate Zygopterideae, far from suggesting that the biseriate forms are primitive, shows, on the contrary, that the most primitive ankyropterid petioles had not yet evolved the biseriate type of branching characteristic of the Clepsydroidae. We may presumably accept Dr. Holden's suggestion that the dichotomous branching of the petiole was probably found also in the ancestral type of the Zygopterideae, and that when this kind of branching was lost, two series of forms, biseriate and quadriseriate, arose. If this was so, the two pairs of rows of pinnae of the Dineuroideae might naturally be regarded as due to a partial retention of the ancestral dichotomy. But, even if we accept this view, that does not settle the question of whether the petiolar bundle was originally more or less radial or a flattened bipolar one. A final consideration of this point is best postponed until after a short discussion of the allied family of the Botryopterideae, especially of *Botryopteris*.

*Botryopteris* is a genus of relatively small protostelic forms. A certain similarity between the emission of the leaf-trace and the branching of the stem (2, 545; 7, 16; 9, 566) has been held to suggest that the family shows the primitive origin of leaf and axis as shanks of an unequal dichotomy. The similarity is most marked in *B. cylindrica* Will. in which the stem branches dichotomously. This species shows some anatomical variation. In the larger mesarch type of cauline stele, regarded by Dr. Bancroft as more primitive than the monarch one with a central protoxylem, the dichotomy is usually unequal (2, 561), and it is this type of cauline branching that the giving off of a petiole most resembles (cf. Scott's fig. 156: 46, 346). But, whereas the protoxylem of the branch is from the first deeply immersed, the leaf-trace is endarch at its origin, though occasionally a few centripetal tracheides, which soon disappear, are present a little higher up. The large size of the petiole and its bundle, compared to the stem and its stele in *B. antiqua* Kidst., in *B. hirsuta* Will. and in *B. ramosa* Will., might be held to suggest that these forms were still near to those in which stem and leaf were not yet fully differentiated, the more



so that *B. antiqua* is geologically considerably the oldest species. Though still relatively large in *B. cylindrica* and *B. mucilaginoso* Kraentzel (27), the petiole and its bundle in these two are smaller compared to the stem and its stele. *B. antiqua* comes from the very base of the Carboniferous; the other species, with the exception of the Lower Permian *B. forensis* Ren., are from the Coal Measures. But caution is necessary. In *B. antiqua* and *B. hirsuta* the petiole is usually larger than the stem and in the former species the leaf-trace and petiolar bundle are from half as large again to twice as large as the cauline stele (23, 3). Even in monarch petioles the bundle is often as large or larger than the axial stele. If branch and leaf were originally part of an undifferentiated dichotomizing thallus, one can hardly suppose that it was the larger shank that became appendicular with reference to the smaller! That reduction in size of the stele has occurred in *Botryopteris* is supported by the small size of the axial stele in *B. antiqua* and by the fact that in *B. cylindrica* the smaller monarch and endarch type of stele seems to have arisen from the larger mesarch type (2, 553). *B. antiqua* was heterophyllous. Some petioles were monarch and were associated with aphlebioid structures, the trace for which was given off quite close to that of the leaf but diverged from it at once to enter the aphlebioid leaflet which was borne on the other side of the stem. Other leaf traces were diarch and in association with these no aphlebioid structures have been observed. No case is known of two diarch traces being given off in succession. The statement that the two kinds of petioles alternate on the stem (21, 537) goes further than the facts warrant. In connection with her text-figure (cf. 3, 1051), Dr. Benson speaks of the "very common monarch petiole bundle" as though diarch forms were less common. Dr. Benson suggests that diarch petioles might have arisen from monarch by an "arrest of branching at a primary phase," and that this view could be extended to the triarch petioles (those of *B. ramosa* and *B. hirsuta*) which might "well bear the same relation to the diarch forms as the latter bear to the monarch forms" (3, 1051). This view is held to be supported by the similarity between a branching diarch petiole of *B. antiqua*, in which the protoxylem of the pinna-trace is individualized some distance below its departure, and a non-branching triarch petiole of *B. ramosa*. It is certainly possible to construct a series starting with the monarch petioles of

*B. antiqua*. From this species the development passes through the diarch forms found in it and in *B. cylindrica*, wherein monarch petioles with the protoxylem spread along the adaxial side sometimes occur (2), to the typically triarch petioles of *B. ramosa* and *B. hirsuta*. In these the protoxylems are at the end of three short adaxial projections. Ultimately, through the newly described *B. mucilaginosa* Kraentzel, with three slightly more projecting protoxylems, the series runs to *B. forensis* Ren. in which the three projections have become long arms and the protoxylems are more numerous.<sup>2</sup> The generally held view that *B. forensis*, the geologically youngest species, is a derivative type, may be accepted as regards the length of the arms (5, 230; 46, 350; 7, 13; 9, 343; 27, M68). But it should be noted that the petiole of *B. forensis* is fundamentally diarch and not triarch. There are only two main groups of protoxylem (Bertrand's "pôles fondamentaux"), one on each side of the middle arm of metaxylem. The numerous other groups of protoxylem are cut off from these and, detaching themselves from the metaxylem for a short space, they work their way backwards along the lateral arms and pass out as parts of pinna-traces (4, 231). They merely represent an extreme example of the early individualization of the protoxylem of a pinna-trace, such as that recorded by Dr. Benson in the branching diarch petioles of *B. antiqua* (3, 1048). It is, however, an open question how the series formed by the remaining petioles should be read. Dr. Benson's view that the monarch type is primitive has been widely accepted (5, 233; 2, 558; 7, 13, 36; 9, 343; 27). Dr. Hirmer, however, regards the monarch petioles as derived from diarch ones by one half of the bundle assuming a median position and the other becoming laterally displaced and entering the aplebioid pinnule. He erroneously attributes a similar view to Dr. Benson (21, 538). The view that the monarch types are reduced, gains some support from the considerations already advanced, tending to show that the stele has undergone reduction in *B. antiqua*; while the heterophylly of this species can hardly be regarded as a primitive character.

As long ago as 1910 Kidston and Gwynne-Vaughan suggested a common origin for Osmundaceae and Zygopterideae and held

<sup>2</sup> Cf. 27; 5, 31, Fig. 26, a figure reproduced by Dr. Hirmer as of *B. tridentata* Felix (= *B. hirsuta* Will.) 21, 535. Fig. 651. On the other hand, Dr. Zimmermann's (original) figure labelled *B. forensis* Ren. (52, 195, Fig. 129) is apparently not a petiole of this species. It may belong to *B. hirsuta* Will. or more probably to *B. ramosa* Will. (34, Pl. 42).

that the petiolar structure of the former was the more primitive. They pointed out that in the Permian *Thamnopteris*, some of the traces possessed, in the neighborhood of the stele, "two immersed protoxylems situated at points more or less apart from the centre of the strand" (25, 469). These traces show an approach in structure to *Clepsydropsis*, the petiolar bundle of which they regarded as primitive for the Zygopterideae. *Thamnopteris* is one of the oldest of the Osmundaceae and now generally accepted as showing, with *Zallesskya*, the most primitive and definitely osmundaceous known stelar structure (46, 278; 21, 596; 52, 211; 7, 136). In *Thamnopteris Schlechtendali* Eichw., the leaf-trace is given off as an oval strand with a single central protoxylem. As this strand passes through the cortex, some of the centripetal tracheides next to the protoxylem cease to be formed, so that a small parenchymatous island arises. This island enlarges tangentially and adaxially and the protoxylems multiply by division until the xylem has become C-shaped with protoxylem groups along its adaxial concavity. Eventually, the trace assumes the same contour as the xylem. Gwynne-Vaughan and Kidston suggested that the changes undergone by this trace in passing through the cortex represent the phylogenetic steps that have led to the development of the monodesmic C-shaped strand, generally recognized as primitive for the Osmundaceae and for the great majority of the Leptosporangiateae (18). They, therefore, regarded a simple monarch petiolar bundle as primitive for the Zygopterideae and the Osmundaceae. Dr. Bertrand accepted this view and extended it to the Botryopterideae (5, 233, 280). For him, Botryopterideae and Zygopterideae were much more closely allied to one another than to the Osmundaceae. He regarded the Botryopterideae as less specialized than the Zygopterideae and more easily comparable to the Osmundaceae. He looked upon the monarch petiolar bundle of *Botryopteris antiqua* as primitive and compared it with that of *Thamnopteris*. The analogy is not so close as he suggested, however, since there seems to be no authority for his statement that the xylem of the trace of *B. antiqua* passes through a phase in which it has an excessively reduced parenchymatous island. He suggested that the primitive petiolar bundle for the three families was either a ring of endarch xylem, enclosing a central island of parenchyma, or a solid strand of xylem with a single central protoxylem. The former hypothesis

has received little or no support but the latter has been accepted in essentials by Professor Bower, among others (9, 577).

To summarize the position: There is a considerable measure of agreement among those whose opinion carries weight that the primitive form of leaf-trace for the ferns generally was a single monarch strand; and there is a larger consensus of opinion in favor of the relative primitiveness in the Zygopterideae of the oval bipolar petiolar bundle as compared with the tetrarch one.

Against these views the following considerations are tentatively advanced.

Whether in the earliest vascular plants the xylem was endarch, mesarch or exarch, it is clear that endarchy, mesarchy and exarchy were all established before the cladode megaphyll was differentiated. The exarch *Gosslingia* is as old or older than the endarch *Rhynia major*. Where a protostele has attained a certain size, it is clear that a single group of protoxylem—and a truly endarch protostele can have but one such group—will hardly prove sufficient to establish an early connection of the conducting xylem with the developing parts, especially with the large leaves of the Pteropsida which, as Dr. Zimmermann remarks (52, 78), overtake and envelop the axis in their development. And in point of fact, the larger protosteles, when devoid of secondary growth, tend to be mesarch or exarch. Moreover, Professor Bower, in his exhaustive account of the morphology of the ferns, has pointed out again and again the presence of parallel developments both in widely separated and in allied form. He says (8, 283): "Further, the progressions in respect of the several criteria are not restricted to any single phylum (of ferns); they are exhibited with a high degree of uniformity in a plurality of phyla, which palaeontology shows to have been broadly distinct from one another in descent from very early periods." Knowing the large size attained by some of the Psilophytales without developing leaves, we are certainly justified in assuming that the megaphyll was differentiated several times independently during the evolution of the ferns, in the wide sense, and in some cases in forms with relatively large exarch or nearly exarch steles. If the megaphyll was originally a but slightly smaller branch of an unequal dichotomy, it is inherently probable that in its early phylogenetic development it would in these cases have carried off several strands of protoxylem. If the stele of the thalloid axis, previous to its

differentiation into stem and leaf, possessed a number, say 10-12, strands of protoxylem in a more or less peripheral position, the weaker branches of the dichotomy might well carry off four of these—or carrying off fewer, might tend by division to restore this number. There is no reason why some of the Zygopterideae, without being in all points primitive, should not have retained in their megaphylls certain characters that belonged to an originally radially constructed branch-system, for instance, the four series of primary pinnae and the approaches to radial symmetry in the foliar bundle of the Dineuroideae. If the biseriate types were primitive, the presence of four arms of xylem and of four main strands of protoxylem in the genus *Ankyropteris* would be hard to explain; but if these characters were a relic of former radial construction their presence in *Ankyropteris* would be more easily explicable. The Zygopterideae are, on the whole, older than the Botryopterideae and much older than the Osmundaceae which have been found below the Permian. Among the Zygopterideae, the Dineuroideae are, as Dr. Hirmer points out, older than the Clepsydroidae, since the structure of the leaf-trace (the free petiole is unknown) in *Asteropteris*, the oldest known zygopterid, makes it almost certain that the leaf branched in the dineuroid manner (21, 506). It is difficult to accept Professor Bower's description of the trace of *Asteropteris* as a "slight modification" (7, 27) of that found in *Clepsydropsis antiqua*. The genera *Stauropteris*, *Diplolabis*, *Dineuron* and *Metaclepsydropsis* all have representatives at the very base of the Carboniferous, while even the later genus, *Etapteris*, has one Lower Carboniferous species (*E. Tubicaulis* Goepp.). The only undoubted biseriate zygopterid recorded from the Lower Carboniferous seems to be *Austroclepsis* (*Clepsydropsis*) *australis* Osborne (50, 198) for of *Zygopteris* (Hirmer's *Protoclepsydropsis*) *Kidstoni* P. Bertrand too little is known to include it in either series, and doubts have again arisen as to the zygopteridean affinity of *Clepsydropsis antiqua*. It is not contended that any zygopteridean bundle known to us is wholly primitive. Indeed, the frequently complicated form of the zygopteridean petiolar bundle suggests considerable specialization. It has been shown by comparison on a wide basis of a number of recent ferns, that the task of carrying forward a sufficient supply of water to the distal pinnae falls in many recent ferns on the incurved adaxial elaborations of the petiolar bundle, while in others

the abaxial parts of the bundle undergo amplification (13). It may well be that the prolongation of the abaxial and adaxial arms of the petiolar bundle beyond the points of departure of the pinna-traces and their curvature in certain Zygopterideae (e.g., *Ankyropteris bibractensis* Ren., *A. westphaliensis* P. Bertrand and *Etapteris Scotti* P. Bertrand) are specializations of this nature. Where differentiation of a thalloid axis into stem and leaf took place in forms with a truly endarch protostele and therefore probably in forms with a small stele, a monarch leaf-trace may well have been primitive. Reasons have, however, been given for thinking that in the Botryopterideae endarchy is a derivative character, associated in reduction with the size of the stele. Moreover, in *Botryopteris Fraiponti* Leclercq, which was not considered with the other species of *Botryopteris*, as it is very incompletely known and may have to be made the type of a new genus, the exarch protostele of the very slender stem (or perhaps of a small branch) appears to have 7-10 projecting groups of peripheral protoxylem. The departure of its trace takes place by the giving off of a chunk of metaxylem, including three of the peripheral groups of protoxylem (33). Thus, the xylem of the leaf-trace, at any rate at its origin, is, like that of the stem, wholly exarch and the similarity of the departure of a trace to an unequal dichotomy of the stem would presumably be closer than in *B. antiqua*, although the leaf-trace is only about a quarter of the size of the cauline stele. It is, however, not known if the stem branched dichotomously. If rightly interpreted, this species would seem to be a very primitive member of the Botryopterideae and would strengthen the case for an affinity between *Botryopteris* and *Grammatopteris*. Dr. Sahni has recently come to the conclusion that the affinity between these two genera, characterized by Scott as close (46, 350), is very remote and that *Grammatopteris* is so closely allied to the Zygopterideae and Osmundaceae that it might be included in either family (43). The arguments against the affinity between *Botryopteris* and *Grammatopteris* are said to rest upon endarchy of the former, the absence of resemblance between the leaf-traces, the habit and phyllotaxy. The habit is hardly of much importance and, as already pointed out, the evidence is against the primitiveness of endarchy in the stem of *Botryopteris*. While the leaf-trace of *Grammatopteris* is admittedly very different from that of a typical *Botryopteris*, its exarchy

may be compared to that of *B. Fraiponti*, a species which, even if it be excluded from the genus *Botryopteris*, appears to be a primitive member of the family. The points of affinity with the Zygopterideae, not shared also with the Osmundaceae, are said to be the differentiated protostele with a stellate core and the bipolar, tangentially elongated foliar bundle, symmetrical in both radial and tangential planes (43, 874). It is, however, admitted that the protoxylems are sometimes and may, perhaps normally, be adaxial; and in the petiole the smaller tracheides frequently lie more towards the adaxial face of the bundle (43, 869). The differentiated protostele is found also in the earlier Osmundaceae (21, 596), while the stellate core seems to have been found only once, near the base of the stem of one specimen and is not very clear there (Cf. 43, Pl. XXXV, Figs. 9 & 10). The affinity of *Grammatopteris* to the Osmundaceae seems clear and it is possible that it should be regarded as the most primitive known member of that group. But there is also an affinity between it and the genus *Botryopteris* and apparently a much closer one than that between *Grammatopteris* and the Zygopterideae though, as the latter family and the Botryopterideae seem to be allied, there appears to be a certain, though a less close relationship between the Zygopterideae and Osmundaceae. The probability seems to be that the three families had a common vascular ancestor which was either a very primitive fern or had progressed a considerable distance along the fern-line. The Zygopterideae constitute the oldest family and seem to have diverged along their own lines, but to have retained in their fronds certain reminiscences of radial organization. The next oldest branch to have differentiated seems to have been that of the Botryopterideae. In them the primary pinnae or the primary branches of the rachis were disposed in two rows and the frond seems to have been dorsiventral, like those of ordinary ferns except, perhaps, in *Tubicaulis solenites* Cotta (Cf. 21, 539 and Figs. 657 and 658). But in the most primitive forms of this line the petiolar meristele probably had more than one strand of protoxylem. In the least ancient of the three families, the Osmundaceae, further removed from both the Zygopterideae and the common ancestor of the three families than the Botryopterideae, these characters seem to have been lost, though the diarch traces occasionally found near the stele in *Thamnopteris Schlechtendalii*, one of the oldest known of the Osmundaceae, may



be the last relics of a petiolar strand that originated phylogenetically as a branch. It is noticeable that most of the early Osmundaceae known to us are large forms, often attaining to the habit of small tree-ferns. In such, the appendicular nature of the leaf at its insertion on the stem might well be emphasized owing to coenogenetic enlargement of the latter.

The somewhat heterodox theory of the course of evolution of the petiolar bundle of the fern frond, advocated above, is in agreement with the now very generally accepted view that the frond was originally a dichotomous branch system; but it is in contradiction with the equally widely held view that in the Coenopterideae, if we trace the petiolar bundle back to its origin from the cauline stele, we shall find it assuming on the whole a more and more primitive form. It seems that a choice must be made between these two views, for it appears illogical to combine them. The view here advocated seems to be historically, *i.e.*, geologically, the stronger. It is not denied that the leaf-trace of the recent Osmundaceae and of the great bulk of the existing Leptosporangiateae is referable to a ground type represented by the C-shaped, monodesmic leaf-trace characteristic of the older Osmundaceae. Some details of this complicated evolution may be open to question; we may even find some forms retaining ancestral features not found in the typical C-shaped trace. But the general outline of the evolution of the leaf-trace and petiolar bundle in the Leptosporangiateae is clear from the work, to mention only a few writers, of Gwynne-Vaughan, C. E. Bertrand and Cornaille, and Professor Bower whose latest book gives a most helpful summary of these multifarious lines of evolution (9, 345). The issue at stake is the relation of this monodesmic C-shaped strand to yet earlier forms of vascular supply.

#### THE MICROPHYLLIOUS OR MEGAPHYLLIOUS NATURE OF THE REMAINING PHYLA

We have now briefly considered the forerunners of leafy vascular plants, the more primitive of the microphyllous types,<sup>3</sup> the forms leading to megaphyllous types and the early typically megaphyllous plants. There remains the question of which of the other phyla were microphyllous and which megaphyllous.

<sup>3</sup> Since the account of the "Yeringian fossil" was written (11a), Drs. Land and Cookson have published a fuller account of this plant and have named it *Baragwanathia longifolia*. Their studies are founded on further specimens, some of them of undoubted Mid-Silurian Age.

In the small phylum of the Cladoxylales, if its morphology may be interpreted in accordance with the structure of the Devonian *Cladoxylon scoparium* Kräusel & Weyland, we would seem to have a group of megaphyllous build, possessing also microphyllous leaflets that may be either repeatedly branched emergences or, more probably, small thalloid branchlets that have become laminar. But, as Dr. Sahni points out (42, 466, foot-note), the reference of *C. scoparium* to the genus *Cladoxylon* is doubtful. The stelar anatomy is closely similar to that of the previously described species of the genus but there is no evidence that these shared the morphological peculiarities of *C. scoparium*.

Many botanists have regarded the Articulatales as microphyllous (1, 9, 21, 23, 52). Goebel, who in the last edition of his "Organographie der Pflanzen" considered only the recent members of the Articulatales, says that in the Equisetaceae the leaves have become smaller and more numerous during phylogeny (14, 1165). This author regarded the large and small leaves of vascular plants as having a similar origin; for him, the size of the primitive leaves of a phylum was not necessarily a decisive factor in the classification of the phyla. Lignier (36), Scott (47) and Dr. Campbell (12), however, look upon the leaf of the Articulatales as primitively branched and more or less of the fern-type. Dr. Campbell's views are in striking contrast to those of other botanists. He thinks it probable that in the ancestors of recent ferns the vascular sporophyte was built up essentially of a large single leaf and of a protocorm or foot. He suggests that it is possible that from ancestral forms resembling the Anthocerotes two series of ferns were developed, one passing through intermediate forms resembling the Psilophytales and "resulting in a broad dichotomously divided frond with terminal sporangia, like *Cladoxylon*, and the other more directly developing the spike-like sporangio-phore of *Ophioglossum* (12). He thinks that *Cladoxylon* and *Hyenia* may resemble the predecessors of modern ferns (presumably of his first series) and suggests that the fan-shaped body (aerial shoot) of *Hyenia* may correspond to the frond of the ferns. He accepts the affinity of *Calamophyton* with *Hyenia* and thinks it quite conceivable that from the latter's type of shoot there arose a typically megaphyllous and primarily monophyllous<sup>4</sup> series; while

<sup>4</sup> Numerous aerial shoots have been shown to be borne on the rhizome of *Hyenia elegans* Kr. & Weyl. (31).

in another direction another series led, through types such as *Hyenia* and *Calamophyton*, to *Asterocalamites* "where the relatively large leaves retain the primitive dichotomous branching" (12, 496). These theories seem to be negated by the very poor geological record of the Anthocerotes and Ophioglossaceae; by certain algal characters of the sporangia of the Rhyniaceae; by the fact that the developmental evidence points to the conclusions: (1) that the ophioglossaceous spike is of pinna nature; (2) that the species of *Botrychium*, with their more freely branched fronds, seem to be more primitive than *Ophioglossum*, the simpler leaved forms of the latter genus being apparently phylogenetically the more modified (7, 87); and by the existence in the Devonian and Carboniferous strata of numerous branch-like fronds and frond-like branch-systems intermediate between the *Psilophyton*-like spiny branch-systems and such fern-like fronds as those of some later Coenopteridaceae, i.e., of species of *Etapteris* and *Anachoropteris*.

The Articulatales, however, do seem to be primitively megaphyllous. Their leaves may well have arisen from ramified thalloid axes which, though themselves dichotomous, were already lateral branches, borne sympodially on a relatively main trunk. An analogy may be found in the lateral fertile dichotomous branches of *Pseudosporochnus* (32). They may, therefore, have been smaller than some of the branch-systems that were converted into fern fronds. Moreover, verticillation, when it was acquired, would tend to reduce the size of the leaves, though in some dendroid Psaroniae the fronds were very large. Professor Bower accepts the simple but relatively wide and pluri-nerved leaves of some forms of *Sphenophyllum* as having arisen by webbing from the geologically older *Sphenophyllum* leaves that were divided into narrow segments, but he holds that dichotomy is the simplest accommodation of form to increasing size and that it occurred also in the microphylls of the Articulatae (9, 149, 160). This is certainly true; apart from *Protolepidodendron Scharyanum* and *P. wahnbachense*, we know of various fossils possessing branched aphlebioid structures that almost certainly originated in evolution as emergences. Professor Bower also argues that the lateral origin of the leaf sheath—itself a highly modified structure—in the embryo supports the microphylls of the group (9, 196); but, as he himself points out, the details of an encapsulated embryo are subject to

plastic differences even within the limits of a single genus (9, 557). The presence of relatively large and forking leaves in a number of the older Articulatales is a stronger argument. Let us consider the four main groups usually included under the Articulatales. In the oldest group, the Mid-Devonian Protoarticulatae, the leaves were once or repeatedly forked and in *Hyenia* attained a length of  $2\frac{1}{2}$  cm. In the Upper Devonian *Pseudobornia*, the only known genus of the Pseudoborniales, the leaves are much larger and so much cut and lobed that when first found, being detached, they were thought to be fern fronds (46, 110). This genus, unfortunately not mentioned by Professor Bower in his latest book (9), presents a very great, one might almost say an insuperable, difficulty to accepting the Articulatales as primitively microphyllous. Dr. Zimmermann, who regards the Articulatales as primitively microphyllous, explains its large leaves by supposing that they originated by a confluence of ultimate ramifications, i.e., by a process essentially similar to that by which he supposes fern-fronds to have arisen. The oldest undoubted members of the two remaining groups of the Articulatales, the Sphenophyllales and Equisetales, seem to be, respectively, the Upper Devonian *Sphenophyllum subtenerrimum* Nathorst and *Asterocalamites scrobiculatus* Schloth., which seem not to be certainly known below the Carboniferous (50, 266). Both have repeatedly dichotomous leaves. From a study of the fertile shoots it would seem, as Professor Seward long ago noted, that the common stock from which Equisetales and Sphenophyllales arose must lie very far back (49, 388; 10, 315). Nevertheless, it is commonly accepted that they and the Pseudoborniales should be associated with the older Protoarticulatae in the same main phylum of vascular cryptogams and the fact that in all of these groups we find branched leaves borne on stems with continuous ribs seems to show a primitive ground plan of forked leaves for the whole phylum.<sup>5</sup> The Lower Devonian *Climaciophyton*, which has been held to be the oldest member of the Articulatales and has been

<sup>5</sup> Drs. Kräusel and Weyland (29, 326) are mistaken in supposing that in a paper published in 1927 (10) the present writer laid great weight on one of their drawings of *Hyenia* as tending to show that the bundles in the Protoarticulatae had the course characteristic of calamitean bundles, i.e., they alternated at the nodes. No such statement was made by the writer in that or in any other paper, though the drawing of theirs in question (28, 134 Fig. 16a) certainly suggests alternation of the bundles, which apparently does not occur.

provisionally compared to the Sphenophyllales (30), might be used in support of the primitive microphyllly of the phylum. The small plants, but a few millimeters long, had irregular axes bearing whorls of three small and relatively thick, simple, sessile leaves. In the absence of fructifications and articulations, it is impossible to say to what phylum *Climaciophyton* belonged. Verticillation of the leaves occurs in the Lycopodiales, e.g., in species of *Lycopodium* and of *Lepidodendron*. In *Drepanophycus spinosus* Krejci sp., a species of the Psilophytales, Drs. Kräusel and Weyland found that the small spine-like leaves were sometimes whorled (as in the examples described by Potonié as *Psilophyton bohemicum*) and sometimes spirally inserted (as in Potonié's *Psilophyton spinosum*) (32). Verticillation of the leaves is, therefore, by itself, no proof that *Climaciophyton* was a member of the Articulatales. It might equally well be a member of the Lycopodiales.

As regards the Noeggerathiales, though little is known of them, we may feel reasonably certain that their leaves were not emergences, though whether they, perhaps including *Barrandeina* and *Duisbergia*, represent an independent pseudomacrophyllous branch or are allied to the Articulatales, more particularly to the Sphenophyllales, is uncertain (11).

Most botanists look upon the Psilotales as primitively microphyllous. Some (26, 839; 40, 159; 9, 145) think that they are probably nearer to the Psilophytales than to any other group, and their complete rootlessness, dichotomous branching and simple vegetative leaves, even if these be held to be somewhat reduced in *Psilotum*, certainly support this view. Scott, towards the end of his life, was inclined to look upon them as aberrant lycopods, which would also make them microphyllous (41, 397). It is, however, possible, especially if the fertile appendages of *Psilotum* and *Tmesipteris* are regarded as forked sporophylls bearing a ventrally situated sporangiophore, that there may be some affinity between Sphenophyllales, Noeggerathiales and Psilotales. In this case, the last-mentioned group would presumably be megaphyllous and their vegetative leaves reduced. The nature of the fertile appendages lies, however, outside the scope of the present essay.<sup>6</sup>

In the above sketch the object has been to fix our attention on certain main lines of evolution which we seem able to discern, at

<sup>6</sup> A short summary of this question is given in 11.

least in part and fitfully. In doing this it has sometimes been necessary to consider forms of doubtful nature or of which we know very little because, although it is undesirable to base theories with a wide phylogenetic bearing on uncertain foundations (29, 326), it is still more undesirable not to attempt to take stock of the position or to do so without considering all the relevant facts. Thus, where anomalous ill-preserved or incompletely understood forms seem to affect a question, the least unsafe course is to consider their supposed structure, bearing in mind the doubtful nature of such evidence. On the other hand, where such types stand more or less alone, or do not seem to bear on the theories under discussion, they have not been considered. But, in conclusion, as some of these forms are of the greatest interest to all students of the Pteridophyta, readers are referred to accounts, by various authors, of *Schizopodium* (19), *Haspia* (29), *Boegendorfia* (17) and *Thallomia* (20).

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## A FRANK LETTER FROM THE EDITORS TO THE READERS OF THE BOTANICAL REVIEW

*Please read it all.* We want to tell you something and we want your advice.

*First, a word of thanks.* We are sincerely grateful to our subscribers for their financial support; without them, the first volume of the Review would have been the last; because of them, the Review looks forward to a long life of usefulness. We are equally grateful to the distinguished botanists who have written for us. We are also appreciative of the help generously given us by our editorial board. Subscribers, authors and advisors have been equally essential. We thank you all.

*Second, a bit of history.* The idea of the Botanical Review grew out of an unfulfilled desire. We wanted to know more about botanical progress but had no time to keep up with the voluminous and widely scattered periodical literature. So we began our plans in November, 1933. At the Boston meeting of the Botanical Society we explained these plans to a score of botanists and found them all enthusiastic. We immediately enlisted the help of a board of editors and according to their advice sent out requests for manuscript. To our surprise and delight scarcely a botanist declined an invitation to write.

But it takes time to write good botanical manuscript, and for a while we were not certain that we could publish our first number in January. Eventually manuscript began to come in, and since those first anxious weeks we have never been without copy.

Our next worry was about subscribers. We felt that our ideas were basically sound and were confident that the Review would soon succeed. But how about the first year? Printing costs money and bills must be paid. We estimated that we might get 200 subscribers the first year

and were prepared to lose nearly a thousand dollars on the first volume.

Our advertising circulars finally arrived and five minutes after they were unpacked we had our first subscriber. We were up to our expected two hundred when the January number appeared. By the middle of March we crossed the five hundred mark and had to increase the size of our edition. By June we passed seven hundred and again had to call for more copies. At the writing of this letter we are at the eight hundred mark and the Review is completely self-supporting.

In the advertising circulars we promised twelve numbers to a volume, containing about 32 pages each, or a total of 384 pages. The first nine numbers alone had 381 pages, so the volume will amount to about 500 pages. You are getting, consequently, 25% more reading matter than you expected. We also indicated that we would print three articles in a number, each with about ten pages. Not one article has been that short. Botanists apparently can not do a subject justice in so few pages or else the topics suggested for treatment are too inclusive. However, a topic too narrow in its scope is not practical. Its usefulness is more restricted and it is isolated from other very closely related subjects. Probably future experience will be about like the past, and you may look forward to articles averaging twenty pages in length.

Several articles by botanists of continental Europe have been submitted in French and German and have been translated by us. Both of us are reasonably proficient in the two languages and we have not hesitated to ask for help from others. We believe we have succeeded in presenting the authors' reviews correctly.

Bibliographic citations have bothered us greatly. Verification of some references has been impossible in New York. In such cases we have had to follow the contributor's copy. Undoubtedly many errors have slipped in. We hope they have not been serious or misleading.

At present there is scarcely an American college or university where botany is taught which does not subscribe to the *Botanical Review*, some of them with several copies. The number of individual subscribers is impressive. A professor in one of our largest institutions, botanically speaking, tells us that the *Review* is the one magazine which every graduate student reads from cover to cover. We are proud of our success.

*Third, a request for advice.* We want the *Review* to please you and to be useful to you. We can not tell how you feel about it if you do not let us know personally. Won't you indicate, on the margin, your opinion about the following points and send it to us along with a renewal of your subscription.

1. What do you think of the length of the articles? Are they too long or too short?

2. Are the articles sufficiently diversified? Is any field of botany receiving too much or too little attention?

3. Is there any particular topic which you would like to have presented? Our advisory editors may think the time is not ripe for discussion of a particular subject or a satisfactory author may not be available. We will, however, give due thought to every suggestion.

4. Do you want more illustrations? Illustrations cost heavily; would you be willing to have the subscription price increased to meet the additional cost?

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6. What do you think of the explanatory notes which we have been adding? Do they help you in reading the article or do you consider them an affront to your intelligence? Their preparation requires considerable thought and time but if they are of value to you they will be continued.

7. Many persons want their addresses changed for the summer months, and even then some report that they do

not receive certain issues. What do you think of publishing nine issues instead of twelve, omitting the three summer numbers? We would then normally publish three articles in each number and you would have 27 articles during the year instead of 24 without any increase in price. This is not suggested to save money; the extra cost of printing a larger volume and the loss of advertising revenue would more than compensate for the slight saving on printing the covers.

8. Is there any other feature of the magazine which you wish to criticize or which you believe could be improved or omitted?

*Lastly, our future plans.* Numerous authors are now at work preparing manuscript for us and we believe the standards of 1935 will be maintained or improved during 1936.

We hope you will continue your subscription. Please understand that the more subscribers, the more reading matter you will receive. Printing and stationery for sending out bills will cost us about fifty dollars. Help us to save your share of that expense by renewing your subscription *now*. The subscription rate is three dollars for the United States and its possessions; three dollars twenty-five cents in New York funds for Canada and all other countries. The slight increase in the Canadian rate has been necessitated by charges incurred in collecting. *We most earnestly urge our foreign subscribers to observe this rate, too, because in view of increased printing costs we must realize three dollars in New York funds for each subscription.* Checks should be made payable to the Botanical Review. Address: Fordham P. O., New York, N. Y.

Again thanking you for your help and advice, we are

Very truly yours,

H. A. GLEASON,  
E. H. FULLING.

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# INSECTS IN RELATION TO PLANT DISEASES<sup>1</sup>

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## INTRODUCTION

Forty-three years ago Waite (78) reported his experiments with the honey-bee and fire-blight of pears, in which he demonstrated for the first time that insects could act as vectors for a plant disease. Today, after forty-three years of intensive research in plant pathology, the degree of importance of bees in the epiphytology of fire-blight is still a debatable question. Rosen (60), on the one hand, contends that the beehive is a source of primary infection and a factor of prime importance in the development of epiphytotics of fire-blight. On the other hand, Pierstoff and Lamb (50), Hildebrand (31) and Thomas and Ark (76) maintain that the pathogen does not survive in the hive and that the hive is of no significance in initiating fire-blight epiphytotics. In a similar way the evidence presented by Tullis (77) in 1929, minimizing the rôle of aphids in the spread of fire-blight as presented in the earlier work of Stewart and Leonard (72) and Merrill (45, 46), leaves this aspect of the problem also in an equally uncertain state. A review of all the work dealing with insects as a factor in the spread and development of fire-blight impresses one with the fragmentary, superficial and conflicting nature of much of the evidence presented.

This situation is cited primarily as an example of the unsatisfactory nature of much of our information on the rôle of insects in the spread and development of plant diseases. It is the writer's opinion that this field of research has not received the attention that its nature and importance justify. This would seem to be an opportune time to review the subject in such a way that its importance and promise as a field of research may be made evident.

<sup>1</sup> Published with the approval of the Director as Paper No. 321 of the Journal Series of the Minnesota Agricultural Experiment Station.

<sup>2</sup> For several years the writer and Dr. A. A. Granovsky, of the Division of Entomology, have been associated in teaching a course on the relation of insects to plant diseases. The writer wishes to express his appreciation to Dr. Granovsky for critically reading the manuscript.

## THE VARIED RELATIONSHIPS OF INSECTS AND PLANT DISEASES

In the minds of many people the association of insects with plant diseases is limited to the simple process of dissemination. Although other aspects have been recognized, the idea of dissemination has almost exclusively predominated in all published literature of a general nature on the subject (6, 54, 55).

To disseminate is to scatter, broadcast or diffuse. To inoculate, as the term is used in plant pathology, is to transport inoculum to a particular part of the plant where infection may result. Wind is a common agent of dissemination but inoculation occurs only when by chance the inoculum falls in the right place. Many insects, because of their habits of visiting certain species of plants and specific organs of those plants, are not only agents of dissemination but also very effective agents of inoculation. The honey-bee and blossom-blight of fruits (78), the sphinx-moth and anther-smut of pinks (8) and pollen-eating flies and ergot of cereals (1) are well known examples of this type of insect inoculation. More recently described associations of this type are those involved in the so-called fruit spoilage of figs (69). Endosepsis (12), souring (13) and smut (49) all depend upon certain fig insects for introducing the respective inocula into the interior of the young fig fruit.

The systemic disease of red clover caused by *Botrytis anthophila* Bond., recently described by Silow (64), is especially illustrative. The spores of this pathogen are transported by the bees that pollinate red clover. The spores along with pollen grains are deposited on the stigma where they germinate. The mycelium of the pathogen grows into the developing seed and persists under the seed coat. When such infected seeds are planted they give rise to systemically infected plants. The fungus grows out into the young flowers, many of which are blighted, and the spores are formed over the surface of the anthers. Bees visiting such blighted blossoms become contaminated with spores and spread the infection to other plants.

All of our higher plants have an effective natural protection against pathogens in the form of cuticle or cork. The entrance or penetration through this external covering by the pathogen has been termed "ingress" by Whetzel (79). Some pathogens are able



to penetrate this protective layer by their own resources but many depend upon wounds. The feeding or oviposition wounds of insects constitute one of the most important avenues of ingress for plant pathogens. The significance of insects as agents of dissemination and inoculation is greatly enhanced when they serve also as agents of ingress. In some cases this aid to ingress overshadows the importance of dissemination and inoculation. The rôle of the woolly aphid in the development of perennial canker of apple trees as described by Childs (19) and McLarty (44) is an excellent example of this type of relationship. These investigators have shown that *Gleosporium perennans* Zeller is strictly a wound parasite and that reinfection of a canker must take place each year. Although plenty of inoculum is present in the canker, reinfection does not occur in the absence of the aphid. The aphids live in the crevices of the canker and make the wounds which are necessary for infection on each succeeding year's growth of callus. Thus the so-called "perennial" canker is actually annual and the apparently perennial character depends entirely on reinfection each year through wounds made by the aphids.

Banfield (3) and Riker and Hildebrand (59) have recently shown that white grubs and several other soil insects play an important part in the opening of infection courts for crown gall, although they are probably of little significance in dissemination of the pathogen.

The vulnerable point in the life history of many pathogens is in their inability to survive the winter period or other adverse conditions of the environment. In recent years many pathogens have been shown to hibernate in the body of insects where they are adequately protected against changes in humidity and temperature, against competition with other organisms and against other adverse conditions. Furthermore, pathogens which hibernate in the body of such insect vectors are in the most strategic position for prompt and successful primary infection in the spring. Rand and Enlows (53, 54) have shown that *Bacillus tracheiphilus* E. F. S., causing cucurbit wilt, survives the winter in the bodies of hibernating adults of the striped cucumber beetle and that this is probably its only method of survival. The recent proof by Poos and Elliot (52) that *Aplanobacter stewarti* (E. F. S.) McCulloch overwinters within the bodies of hibernating adults of the flea beetle, *Chae-*

*tocnema pulicaria* Melsheimer, has thrown much light on the epiphytology of the wilt disease of sweet corn. Similar survival in overwintering adult insects has been demonstrated for the blue-stain fungi, *Ceratostomella ips* Rumbold and *Tubercularilla ips* L., O. and C. and the pine bark beetles *Ips pini* Say and *Ips grandicollis* Eichh. by Leach, Orr and Christensen (40). The pathogen of the Dutch Elm disease, *Ceratostomella ulmi* (Schwarz) Buisman, apparently survives in a similar manner in the bodies of *Scolytus multistriatus* Marsh and *S. scolytus* Fab. (4, 27, 28, 43). It is also of considerable significance that plant pathogens may survive within the puparium of certain insects during the process of metamorphosis and be present in considerable numbers in the intestinal tract of the newly emerged adult. This has been demonstrated by Petri (48) working with the olive fly, *Dacus oleae* Rossi, and the olive knot pathogen, *Bacillus savastanoi* E. F. S., and by Leach (38, 39) working with the seed-corn maggot, *Hylemyia cili-crura* Rond. and *Bacillus carotovorus* Jones. In the case of the olive fly the bacteria are also transmitted internally through the eggs but the eggs of the seed-corn maggot, although often surface contaminated at oviposition, are always internally sterile.

#### INSECTS AND VIRUS DISEASES

In recent years striking progress has been made in the study of the virus diseases of plants (68). A formidable list of diseases and their insect vectors has been built up. An analysis of the accumulated data shows that there must be extremely varied and complicated biological relations between some of the viruses and their insect vectors. Up to the present time, however, relatively little exact information is available about the more intimate biological relations between insects and viruses (67). The surface has been merely scratched and it would be decidedly rash to attempt to generalize extensively on the data available. Nevertheless, some recent work is indicative of results that may be obtained when a concerted effort is made on this fascinating but perplexing problem. Only a few of the more significant facts will be mentioned here.

Many virus diseases are highly infectious and are easily transmitted by mechanical means. Others apparently can not be mechanically transmitted but are readily transmitted by certain rather

specific insect vectors. At the present time about 25 reasonably well studied diseases fall in the latter group. These are said to be biologically transmitted and an incubation period of varying length within the body of the insect vector is required in all cases. These "biological" insect vectors include about 15 leaf hoppers, 10 or 12 aphids, 2 species of thrips and 1 white fly. It is the nature of this biological association between virus and insect vector that especially needs elucidation.

Interesting and suggestive is the recent work of Storey (74, 75) who showed that in *Cicadulina mbila* Naude, the vector of maize streak, the ability to transmit the virus is inherited as a simple dominant sex-linked Mendelian factor. Those individuals inheriting the ability to transmit the virus were termed "active." Individuals not inheriting the ability to transmit the disease ("inactive") did not become viruliferous by feeding on infected plants but were made infective by directly inoculating the insect with the virus by means of a micropipette. Evidence was presented to show that in "active" insects the virus entered the intestines by the mouth and passed through the intestinal wall into the blood, but that in "inactive" insects the virus could not pass through the intestinal wall. When the virus was injected into the blood with a micropipette, the insect became viruliferous and was able to transmit the virus to plants. Transmission was somewhat more irregular than with normal "active" insects and such artificially activated vectors usually became non-infective after varying periods of time, while normal "active" insects remained infective until death.

When "active" insects were fed on infected plants the virus could be demonstrated in the blood after 6 to 9 hours, while the insect could not transmit the virus to plants before 12 to 15 hours after feeding on infected plants. This is added evidence that the blood is the vehicle by which the virus is transported to the salivary glands.

Of equal interest is the recent report by Fukushi (29) that a virus causing a mosaic of rice and transmitted by the leaf hopper *Nephotettix apicolis* Motsch is transmitted from generation to generation of the insect vector through the egg. If this work is verified, it will be the first definitely proven case of the transmission of a plant virus through the egg of its insect vector.

The biological association of viruses with thrips presents another significant variation. Samuel and Bald (61) working with *Frankliniella insularis* Frankl. and spotted wilt of tomato and Linford (41, 42) working with *Thrips tabaci* Lind. and pineapple yellow-spot showed that adults do not become viruliferous when fed on diseased plants but that young larvae become infective after a period of ten days and that they remain viruliferous through pupation. Thus adults are infectious only when reared from viruliferous larvae.

The nature of the relationship between virus and insect vectors offers considerable promise as a criterion for virus classification. Elze (22), Storey (73) and Johnson and Hoggan (34) have all published schemes of classification in which such relationships play a prominent part. Kunkel (37) considers insect relationships as one of the most useful criteria for differentiation and classification of viruses. A better understanding of the nature of the biological relationships between viruses and their insect vectors obviously will be an important step in the solution of the virus problem.

#### INSECTS AND NON-PARASITIC DISEASES

When considering insects in their relation to plant diseases one must not overlook those non-infectious diseases that follow insect feeding but are not caused by any known microorganism or virus. One of the best known diseases of this type is the so-called hopper burn of potatoes or yellow top of alfalfa shown by Ball (2) and Jones and Granovsky (35) to be caused by the feeding of the potato leaf hopper *Empoasca fabae* Harris.

The earlier work of Granovsky (30), Monteith and Hollowell (47), Poos (51) and others (23, 26) led to the suggestion that the probable cause was a diffusible toxic substance or enzyme injected into the plant by the leaf hopper. In 1930 Granovsky (30) reported that microchemical tests revealed a greater accumulation of starch grains and sugars in affected tissues. There was also a clogging of vascular bundles and complete disorganization of the phloem region of severely injured tissue, phenomena held to be due to enzymic secretions by leaf hoppers in the course of feeding. Later investigations by Smith and Poos (66), Smith (65) and Johnson (33) have verified the histological changes reported by Granovsky (30) but these authors consider it improbable that the

leaf hopper injects a toxic substance. They suggest that the over-accumulation of carbohydrate products of photosynthesis above the points of feeding are sufficient to cause the symptoms usually attributed to a diffusible toxic substance.

In contrast to hopper burn of potatoes is the psyllid yellows described by Richards (57) and Richards and Blood (58). This striking disease is caused by the feeding of a considerable number of the psyllid, *Paratriosa cockerelli* Sulc. The disease is systemic and causes profound abnormalities in the physiology of the plant. In addition to other striking symptoms, tuberization is inhibited, often to a degree sufficient to cause a total loss of the crop. There is little direct evidence as to the nature of the injury. Although Binkley (5) has designated the trouble as a virus disease and Shapovalov (63) has reported tuber transmission, their results do not agree with the more extensive work of Richards and Blood (58). The results obtained by the latter workers point to the presence of some toxic substance introduced by the insects while feeding.

The mealy-bug wilt and green-spotting diseases of pineapples, originally described by Illingworth (32) and studied further by Carter (15, 16), are perhaps the most interesting diseases of this type. According to Carter (15) mealy-bug wilt is caused by a phytotoxic secretion of the mealy-bug *Pseudococcus brevipes* Ckl. Plants are wilted suddenly only when the initial population of mealy-bugs is relatively large. When the initial population is small the plant builds up an antitoxic reaction and does not wilt until the population is excessively large. The toxicity of the insects is influenced by the host plants previously fed upon.

The green-spotting disease is caused by the same insect but is distinct from wilt (16). It also is caused by a phytotoxic secretion of the insect. The toxic principle in this case is correlated with the presence of certain symbiotic rod-shaped bacteria in the mycetome of the mealy-bug. The symbiotic bacteria are not present in all insects but when present may be transmitted from mother to young. The presence of the symbiotic bacteria and the toxicity of the insect depend on the plants upon which the insect has fed. After the insects have fed for two generations on *Panicum barbinode* the bacteria are lost and the insects become non-toxic (17, 18).

The almost universal presence of mycetomes and their intracellular symbiont in Homopterous plant-sucking insects as reported by Buchner (10) and others makes this work very significant and justifies further studies of these symbionts in relation to insects associated with plant disease both infectious and non-infectious.

THE BIOLOGIC AND EVOLUTIONARY SIGNIFICANCE OF THE  
ASSOCIATION OF INSECTS AND PLANT PATHOGENS

Research workers in phytopathology have, as a rule, considered insects merely as one of several agencies of inoculum dissemination to be disposed of in the ordinary routine study of a disease. It is the opinion of the writer that the relation of insects to plant diseases is too important and too complex to be handled in such a summary manner. The association of insects with plant pathogens is, for the most part, not a mere haphazard matter of chance but rather a highly organized relationship which, in many cases, may have arisen out of a close association of insects and microorganisms over a period of 50 million years or more. It is a definite biologic phenomenon of evolutionary significance and has broad biologic and economic implications. As such it deserves more than routine attention. It deserves the same type of sustained and coordinated thought and effort that has been given to other phytopathological problems of fundamental nature.

As a biologic phenomenon the association of insects and plant pathogens is in many respects similar to that of entomophily, i.e., insect pollination. All students of botany or of entomology are familiar with the remarkable evolutionary adaptations of insects and flowers. It will not be necessary here to describe the various details of these adaptations. Along with the morphological adaptations on the part of both insects and flowers there has been built up a constant and regular association of insects and plants that is mutually beneficial to both parties of the association. Let us consider how this association came about. It is agreed by most, if not all, students of plants that our most primitive flowering land plants were wind pollinated. The pollen grains were disseminated by the wind and pollination was accomplished only when, by chance, a pollen grain accidentally fell upon a stigma of the proper species. This necessitated the production of enormous quantities of pollen and resulted in a tremendous waste of materials. That this was

true is abundantly verified by the geological records of the plants of the Carboniferous age. But in one fortunate period long ago, insects discovered the food value of pollen and perhaps the associated secretion of nectar and began to make regular visits to the source of supply. In this way the pollen was transported with very little loss to the stigmatic surface of the proper plant. This association had a survival value for both insects and flowering plant and consequently has persisted throughout the ages and has developed to a state of very high efficiency. A little reflection will show that the principle underlying this association is essentially the same as that underlying that of insect dissemination of plant pathogens. Some pathogens are disseminated only by wind. In such cases relatively large quantities of inoculum are produced but only small amounts reach an infection court and survive. Other pathogens have become associated with insects which transport them or their spores to the required infection court with a minimum of waste and a maximum of efficiency, especially when the insects provide also a means of ingress and of protection against the elements. This type of association, like insect pollination, also has a survival value and numerous cases have persisted with remarkable adaptations on the part of both insect and pathogen. Although survival value does not predicate benefits by both insect and pathogen, there are numerous cases of striking mutual benefit and interdependence.

But it would not be fair to drop the comparison here for there are other important factors which have influenced the complicated adaptations associated with entomophily. With the development of insect pollination the nature of the process led to an increase in cross-pollination in comparison with self-pollination. Cross-pollination increased the frequency of hybridization and this in turn stimulated variation, a primary factor in evolution. Thus insect pollination by stimulating hybridization and variation has speeded up the development of entomophily itself. This factor, so important in evolution, is apparently lacking in many of the associations of insects and plant pathogens and may account for the fact that the latter association is less obvious than entomophily.

But recent investigations have shown us that the factor can not be entirely removed from the picture. With the discovery of heterothallism in fungi by Blakeslee (7) and its further elucidation



by Kniep (36), Buller (11), Stakman *et al.* (71) and others the significance of hybridization in the evolution of fungi is more clearly discernible.

Brodie (9) has shown how flies are agents of "diploidization" in the fungus *Coprinus lagopus*, a process fundamentally analogous to insect pollination of higher plants. *Coprinus lagopus* is a heterothallic fungus. Each basidiospore formed on the "inky-cap" mushroom, upon germination, forms a homosexual mycelium which is unable to reproduce the mushroom stage until it has been fertilized or diploidized by fusion with a mycelium of the opposite or complementary sex. Each of these mycelia produces spores in drops of a sticky solution which is attractive to flies. Flies, by feeding on these droplets, transport spores from one mycelium to another. The spores promptly germinate and fuse with mycelia of opposite sex resulting in diploidization and the formation of the mushroom stage. Dowding (21) also has pointed out a similar association between *Ascobolus stercorarius* (Bull.) Schröt. and mites and flies.

A similar case has been revealed by the work of Craige (20) on *Puccinia graminis*, the well known wheat-rust pathogen. In this heterothallic fungus diploidization occurs on the barberry leaf. Flies, attracted by the drops of sugary solution formed on the bright orange colored pycnia, often transport the pycnospores from one pycnium to the other. Thus insect transportation of pycnospores increases the chances of hybridization. The large number of physiologic forms which occur in this pathogen can be explained to a large extent by the hybridization that occurs on the barberry (71). Is it merely a coincidence that such specialized adaptation for insect "pollination" of a fungus, with resulting hybridization, should be found in one of the most highly developed plant pathogens and one in which physiological specialization has reached such a high state of development?

#### SYMBIOSIS BETWEEN INSECTS AND MICROORGANISMS AND ITS SIGNIFICANCE IN PLANT PATHOLOGY

In the preceding discussion we have been concerned primarily with the association of insects and those fungi and bacteria which are plant pathogens. But the association with insects is by no means confined to pathogenic microorganisms. In all probability

the association arose first among the non-pathogenic forms. The work of Paul Buchner (10) and his students, summarized in his book "Tier und Pflanze in Symbiose" published in 1930, has shown us the astounding universality of symbiosis between insects and microorganisms and the high degree of development it has reached. Buchner's investigations are of great significance to the student of insects in their relation to plant diseases. They have shown that symbiosis with microorganisms is to be found in nearly all orders of insects and that in some groups such as those feeding on plant sap and on wood it is almost universal. In several cases the symbiotic microorganisms have proven pathogenic to plants. His studies of the subject have yielded sufficient evidence to justify the conclusion that the interrelations of insects and symbiotic microorganisms are as complex as, and of evolutionary significance equal to, those between insects and flowering plants. The almost universal occurrence of symbiosis between insects and microorganisms in general, coupled with the numerous striking cases involving plant pathogens, in the writer's opinion, makes it imperative that we recognize its importance and significance in the study of plant diseases.

THE POSSIBLE RÔLE OF INSECTS IN THE ORIGIN OF NEW DISEASES  
AND THE EXTENSION OF OLD ONES

There is one other aspect of the association of insects and plant diseases that is generally overlooked. This is the possible and probable rôle of insects in the sudden appearance of new and destructive plant diseases. We are accustomed to thinking of plant diseases largely in terms of the pathogen, an organism of ancient lineage which, in common with other plants, has had a long and slow process of evolution during which its efficiency as a pathogen has also evolved. But a disease is not an organism. It is a biological process. It is the result of the interaction of two or more organisms, influenced by a host of other factors. When it is considered in this light we are faced with the possibility that many of our plant diseases may be of relatively recent origin. For the purpose of illustration let us consider the Dutch Elm disease. This disease was first recognized in South Holland in 1919 (62, 70). There seems to be considerable evidence that it has spread from this center to most of the countries of southern and western Europe

and it has been introduced also into the United States (43). Its origin is obscure but the general assumption seems to be that it may have been introduced into western Europe from some other part of the world. The writer does not wish to challenge this assumption, for he has no evidence to offer, but it is suggested that as a disease in the sense of a biological process it may be entirely possible that it had its origin in western Europe within relatively recent years. The bark beetles that constitute the principal agent of dissemination of *Ceratostomella ulmi* were known in Europe and America for many years prior to the appearance of the Dutch Elm disease (25). At the present time there are large areas in which the beetles occur but where the disease is absent (25), but these areas appear to be rapidly diminishing. This fact, although by no means conclusive, would seem to indicate that the association is probably not one of long standing. *Ceratostomella ulmi*, although a virulent pathogen on elms, grows well as a saprophyte on dead wood or bark. It apparently is not well adapted to wind dissemination and apparently can infect only through wounds. In the absence of a suitable insect vector it would probably exist chiefly as a saprophyte on dead wood and perhaps be disseminated from one dead tree to another by insects inhabiting dead wood. As such it would attract no attention and would scarcely justify consideration as a disease. But when it becomes associated with the Scolytus bark beetles, which prove to be ideal vectors, there suddenly appears a very destructive disease. Not only this, but the elm bark beetles, which by their nature can breed only in weakened or dying elm trees, find the association to their advantage. By transporting the spores of *Ceratostomella ulmi* and introducing them into feeding wounds they provide themselves with an abundance of dead and dying elms and thereby rapidly increase in number. More vectors, more disease, more dead trees, and more vectors! A vicious circle that results in an epiphytotic disease that threatens to destroy one of our most valuable trees. This order of events in the origin of the Dutch Elm disease is purely theoretical but it is well within the realm of possibility and deserves consideration. It is suggested here primarily to illustrate one of the many significant aspects of the association of insects with plant diseases. Many insects as well as plant pathogens are, or until recently have been, relatively local in their distribution. With in-

creased commerce and travel throughout the world they are becoming more widespread in spite of all of our efforts to control them. With promiscuous intermingling of insects and pathogens would it not be surprising if new combinations and associations were not formed in which an insect and a pathogen (neither of which alone may be conspicuous or of economic importance) working together may give rise to a destructive disease?

The invasion of new areas by insects may also profoundly influence the distribution of certain well known destructive diseases. Curly top of sugar beet, for example, is limited in its distribution in North America to the arid regions of western United States. According to Carter (14) this is true because the insect vector (*Eutettix tenellus*) can not thrive in the more humid regions. It recently has been shown by Fawcett (24) that curly top in Argentina is spread by another leaf hopper, *Agallia stictocollis*. This insect is said to thrive in regions of relatively high humidity. If it should be introduced into the United States, it is very probable that the range of curly top would be greatly extended.

#### THE FUTURE DEVELOPMENT OF RESEARCH IN THE FIELD OF INSECTS IN RELATION TO PLANT DISEASES

In 1920 Rand and Pierce (55) published an article entitled "A coordination of our knowledge of insect transmission in plant and animal diseases." This was the first general discussion of insects in relation to plant diseases to be published. As a result of the interest created by this article the American Phytopathological Society and the American Association of Economic Entomologists at their annual meetings in Toronto, Canada, on December 31, 1921, held a joint symposium on "Insects as disseminators of plant diseases" (56). One of the results of this symposium was to make more evident the need for closer cooperation between entomologist and plant pathologist. In spite of this evident need the situation today leaves much to be desired.

The lack of adequate cooperation is frequently but not always due to the human and personal element. The causes may be administrative or political; and still others may be matters of tradition. It is the writer's opinion that the wide gap separating research in the two fields of plant pathology and entomology is mainly traditional in nature.

Although entomology and phytopathology have one large problem in common, the protection of plants, the two fields of science have widely divergent outlooks. Not so many years ago there was a single branch of science known as biology and it was possible for one man to obtain a fair knowledge of the entire field. But this is not true today. Early in its history biology became sharply divided into the two fields of botany and zoology. Each of these fields has been divided and subdivided almost *ad infinitum*. Today two young students of biology start out together but very soon they come to the parting of the ways, one to become a zoologist, the other a botanist. After many years of training one becomes an entomologist, the other a plant pathologist, and they meet again both faced with the problem of plant protection. They realize the necessity of coöperative effort and make an attempt to coöperate, but lo! and behold! "They don't talk the same language." Their training has been too widely separated and too specialized. This condition of affairs has resulted in the neglect of a large field of information that is vital to the problem of plant protection.

The writer does not think that this difficulty can be avoided by the expedient of coöperation alone. Before these two workers can coöperate effectively they must be able to understand and speak a common language. Coöperative work is sometimes attempted on the principle of strict division of labor in which all "entomological" work is done by the entomologist and all "plant pathological" work by the plant pathologist. This type of coöperation is doomed to failure. In the problem of insects in their relation to plant diseases no such division is possible. For the greatest success the invisible, though very real, wall separating the two fields of research must be broken down. This may be rather difficult but it can be done. A first step in this direction would be a modification of our educational procedure so that research workers would be given the training and viewpoint necessary for the solution of the problem in hand. The worker must have a thorough knowledge of the essentials of both entomological and microbiological technic. He must have in his own mental make-up the viewpoint of both entomologist and plant pathologist. When these qualifications are combined in one man many of the difficulties of coöperation will be avoided.

A second step would be a liberalization of the narrow professional viewpoint which in effect often hangs out a sign reading: "This is the phytopathological field; all entomologists keep off," or "This is the entomological field; phytopathologists encroach at their own risk." Such a viewpoint may simplify some of the problems of organization but it is not conducive to the solution of these neglected borderline problems. The necessity of well defined fields of research, with corresponding responsibility and authority, is well recognized. Such responsibility and authority is necessary, not only for effective administration, but also for the existence of the guild spirit which is so important in scientific research. Nevertheless, when attempts are made to draw too sharp a line between related fields of activity many problems of vital importance and significance are usually neglected.

Even when coöperative relationships are cordial the sharp line separating the two traditional fields often results in a shifting of responsibility that is not conducive to efficiency. The plant pathologist with his vague knowledge of insects finds it easy, when faced with an insect-disease complex, to shirk his responsibility by saying, "That's up to the entomologist." Likewise the entomologist finds it equally easy to shy away from any problem that might involve a mycological or bacteriological study.

The importance of insects in relation to human diseases has led to the development of the rapidly growing field of medical entomology. It is manned by investigators trained in the fundamentals of both entomology and medical science. They have for their goal the solution of a particular kind of problem, namely, the rôle of insects in the spread and development of human and animal diseases. It would seem that the time is ripe for a similar development in the field of plant pathology.

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# THE BOTANICAL REVIEW

VOL. I

DECEMBER, 1935

No. 12

## CYTOLOGY OF CEREALS<sup>1</sup>

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The cereals included in this review are those most commonly spoken of as "small grains," wheat, rye, barley and oat. The first three are members of the tribe *Hordeae* and oat is a member of the tribe *Aveneae*, of the grass family. The chromosome number is 7 or a multiple of 7. Wild forms, known commonly as grasses, are also discussed when used as co-parents with these cereals in hybridization.

Summaries of chromosome numbers (23-26, 114, 115) quickly reveal that purposeful effort in cereal cytology began with the works of Sakamura (97) in 1918. The pioneer work of Kihara (51) in 1919 suggested the possibility that not only chromosome numbers in pure species, but chromosome behavior at maturation divisions in anthers and ovaries might give valuable information toward the solution of puzzling problems in taxonomy and genetics. An increasing wealth of material is made available to the cytologist through greater success in obtaining intergeneric crosses, earlier thought impossible. Weed gardens around the world are scrutinized ever more closely for any wild and little-known cereal or grass that might contribute in some small way toward a better understanding of how nature builds her species and also, possibly, toward the development of economically desirable forms.

Certain improvements in cytological technique, especially in the smear technique, have encouraged more intensive study in chromosome morphology. Cereal chromosomes were considered to be of fairly large size, somewhat long and slender but unfortunately too similar. Kagawa (41), Avdulov (2), Emme (18), Lewitsky (66), Senjaninova-Korczagina (105), and others have devised methods

<sup>1</sup> Contribution No. 52 from the Botany Department of the State College of Washington.

for more detailed observation as to exact size and form, the latter including constrictions, satellites (appendages) and fiber attachments. Some chromosomes of a genom show outstanding individual characteristics and are easily identified, others must be classed in groups of morphologically like chromosomes. One genom, or set of 7, will differ little or much from another set of 7 in respect to individual chromosome morphology, and the degree correlates usually rather favorably with taxonomic and genetic conclusions as to relationships of the plants compared.

Darlington (12-14), Hosono (35), Mather (72), Sax (102), Spier (37, 106), and others have added to the study of chiasmata (locations of supposed chromatid partner exchanges) and their relations to problems of chromosome pairing, and of the, not too well understood but extremely crucial, maturation divisions in general.

#### WHEAT

The greater part of cytological work in "small grain" cereals has centered about the genus *Triticum*. Much of this work involves also the genera *Secale*, *Aegilops*, *Agropyron* and *Haynaldia*. Taxonomically, the five genera commonly appear together in the sub-tribe *Triticinae* (39) under the tribe *Hordeae*. The possibility of inter-crossing in these genera substantiates the relationships. One species, for instance, has masqueraded under the name of each of four of the genera, as *Triticum villosum* (L.) M. B., *Secale villosum* L., *Agropyron villosum* Lk. and *Haynaldia villosa* Schur. In cytological literature it has been designated as *T. villosum*, but there is a tendency also to retain the genus name *Haynaldia*. Oehler (88) states his preference for *Haynaldia villosa* because several easily recognizable morphological characters distinguish the plant quite definitely from the species of the other four genera.

The chromosome number is known for all 14 to 16 species, subspecies, or races of *Triticum*. The gametic chromosome numbers 7, 14 and 21 coincide with the three taxonomic groups as presented in Flaksberger's classification (20), Percival's wheat monograph (89), Schiemann's general discussion of cereals (104), Watkin's (123) critique on wheat, and other sources. The 7-chromosome or einkorn group is small, including 2 or 3 species with approximately twenty varieties, sometimes referred to as "small spelt."

The 14-chromosome or emmer group embraces 7 to 9 species yielding several hundred forms. The group includes not only "spelts" with brittle rachis and covered grains but several species like *T. durum*, the macaroni and cracker wheat, with grains that thresh naked. The 21-chromosome or vulgare group comprises 4 species which illustrate admirably the results of allopolyploidy, *i.e.*, the combining into one new composite species the genomes (basic chromosome sets) of less closely related species. Vulgare wheat is probably a composite of three such primary 7-chromosome genomes (diagram 1), each of the three genomes differing more or less as to the inherent morphology and physiology of its individual chromosomes from each of the other two genomes. This tripling of building blocks allows ample material, through interplay of dominant and recessive genes, for the rise of various morphological and physiological types, the existence or life of the variants being, in turn, safeguarded by the triplicate genomes. Watkins (123) states that Percival at Reading, England, yearly grows over 1300 varieties of *T. vulgare* alone. The 21-chromosome wheats, with the exception of *T. spelta*, have naked-threshing grains. The varieties of *T. vulgare* of this group are the outstanding bread wheats of the world.

Cytological data are available for over 80 *Triticum* crosses, involving over 40 species or race combinations (1, 23-26, 35, 51, 57, 72, 91, 110, 118, 119, 125). Hybrids within the same chromosome group show comparative regularity of chromosome pairing at meiosis or maturation division, indicating compatibility approaching that of the pure species. The two chromosomes of a pair are usually attached together at both ends forming a ring. Rod-like figures resulting from one end attachment increase in number with the distance of the cross. Unpaired chromosomes in near crosses are found with slightly greater frequency than in the parents. With increase in polyploidy, irregularity in pairing, even in selfed species, increases. Thompson and Robertson (113) observed irregularities, as univalents, in .5-3.4% of pollen mother cells in species of the 14-chromosome group and in 4-4.1% in species of the 21-chromosome group. The frequencies increased in hybrids of these species, varying in the 14-chromosome group from 1.8-26.9% and in the 21-chromosome group from 12.5-42%. Hollingshead (33) found in varietal crosses of *T. vulgare* 5.2-39.1%.

The most common number of univalents was 2 but as many as 8 occurred. Hosono (34) observed rings or irregular chains of 4 chromosomes with conspicuous frequency in some of the 14 chromosome crosses. *T. dicoccum*, in certain hybrid combinations, seems especially conducive toward this irregularity.

Fertility of  $F_1$  hybrids within a chromosome group is usually high, frequently approaching that of the parents (34). An outstanding exception (assuming the exclusion of *villosa* from *Triticum*) is produced by introducing into the crosses the wild wheat, *T. Timopheevi*, recently discovered in Georgia, U. S. S. R. Kihara and Lilienfeld (57) report for these crosses a fertility of 0–4.8%. The small number of ring pairs and other irregularities at meiosis agree with the low fertility. In crosses with the 7-chromosome wheat, pairing approaches 7 which is normal in the triploid cross. It seems that *T. Timopheevi* possesses the *A* genom of the 7- and 14-chromosome wheats but its second genom seems not highly compatible with the *B* genom of the other 14-chromosome wheats. This fact may explain its failure, as yet, to hybridize with the 21-chromosome wheat assumed to contain the genomes *A B C* (diagram 1). Kihara and Lilienfeld suggest the genomes *A G* for *T. Timopheevi*. Cytological findings are in agreement with morphological departures of the plant.

Hybrids between the chromosome groups are characterized by high sterility. The number of pairs at meiosis approaches that of the parent with the lowest chromosome number. The number of probable pairs is most complete, and ring pairs are more predominating in the 21- $\times$ 14-chromosome hybrid (table 1); this hybrid is partly self-fertile (98). The 14- and 21-chromosome groups are apparently more closely related to one another than either of them is to the 7-chromosome group. In other words, vulgare wheats have originated comparatively recently by the crossing of a 7-chromosome form with an emmer which had, in turn, originated through the crossing of two 7-chromosome wheats and had been in existence long before the hybrid now known as *vulgare* arose (diagram 1).

Wild grasses of the genus *Aegilops* have played an extensive rôle in intergeneric hybrids of wheat. Senjaninova-Korczagina (105) writes: "The genus *Aegilops*, which in some of its morphological characters approaches wheat, has proved rather poly-



morphous. Its species, in the majority of cases showing transitional forms, are difficultly classified and grouped into sections. These difficulties of classification have evidently found their expression in the great differences between the systems existing for *Aegilops*. P. M. Zhukovsky, in his monograph on *Aegilops* (1928), establishes 20 species which he arranges in 9 sections. A. Eig (1929) counts 22 species arranging them into 6 sections."

The chromosome numbers as in wheat are 7, 14 and 21. Attempts to make the chromosome groups coincide with taxonomic divisions have not been entirely successful. At least two species have varieties with 14 and 21 chromosomes. Unlike *Triticum*, the 7-chromosome *Aegilops* species are comparatively numerous, approximating 13. There are about 10 species with 14 chromosomes, and 3 or 4 species with 21 chromosomes.

A study of cytological reports (1, 23-26, 42, 43, 56, 75, 90) of 38 hybrids in *Aegilops*, involving 20 different species combinations, forces the conclusion that the genus presents a cytological complexity quite paralleling that of its taxonomy. With rare exceptions, hybrids within either the 7- or the 14-chromosome group have been found to be highly sterile. Pairing at meiosis is spasmodic, ring pairs do not predominate, complexes of 3, 4 or more chromosomes are common. Hybrids between the chromosome groups show but little more irregularity. The study of chromosome charts presented by Senjaninova-Korczagina (105) shows that while species may have many morphologically homologous chromosomes in common, frequently a larger or smaller number is different. On the basis of the types of morphologically similar chromosomes, the species of *Aegilops* may be grouped into sections allying themselves usually with natural taxonomic sections but not with the 7-, 14- or 21-chromosome groups. Within the groups of greatest morphological homology of chromosomes, should be obtained the more meiotically regular, and possibly even a few fertile hybrids; this is exemplified by the hybrid *Ae. speltoides* ( $n=7$ )  $\times$  *Ae. Auchera* ( $n=7$ ), as reported by Kihara and Lilienfeld (56).

Cytological data (1, 8, 10, 23-26, 52, 56, 59, 70, 73, 90, 91) are available for more than 120 *Triticum-Aegilops* crosses comprising over 60 different species crosses. These intergeneric crosses are highly sterile in any chromosome combination (table 1). Meiotic conjugation is feeble, as shown by rarity of ring conjugants, and is

TABLE 1. General summary of chromosome conjugation in  $F_1$  of cereal hybrids. The number (or numbers) in each rectangle represents the most frequent number (or numbers) of chromosome pairs. Numbers occurring in a very high percentage of cells are underlined. The parents in each respective cross are named at the extreme left of the horizontal column and at the extreme top (in abbreviation) of the vertical column in which the particular rectangle is located. The number of unpaired chromosomes may be obtained

	Tr. 7	Tr. 14	Tr. 21	Haynal- dia 7	Hynltr. 21
<i>Triticum</i> <sup>7</sup>	<u>7</u> ■ ●				
<i>Triticum</i> <sup>14</sup>	4-7 □ ●	<u>14</u> ■ ●		<u>0</u> □ ○	
<i>Triticum</i> <sup>21</sup>	3-7 □ ○	<u>14</u> ■ ●	<u>21</u> ■ ●		
( <i>Haynaldtricum Turgidovillosum</i> ) <i>Triticum Turgidovillosum</i> <sup>21</sup>		12 □ ●	14 □ ●		20-21 ■ ●
<i>Aegilops</i> <sup>7</sup>	2,3 □ ○	1-7 □ ○	4,7 □ ○		
<i>Aegilops</i> <sup>14</sup>	0-7 □ ○	<u>0-6</u> □ ○	0-4† □ ○	<u>0</u> □ ○	
<i>Aegilops</i> <sup>21</sup>		0-6 □ ○	4-7 □ ○		
<i>Aegilotriticum</i> <sup>28</sup>		13 ●	14 ●		11,12 □ ○
<i>Secale</i> <sup>7</sup>		<u>0,1</u> □ ○	<u>0</u> □ ○		
<i>Secalotriticum</i> <sup>28</sup>					
<i>Agropyron cristatum</i> <sup>14</sup>					
<i>Agropyron glaucum</i> <sup>21*</sup>			2-3		
<i>Agropyron glaucum</i> <sup>21(?)</sup>			14		
<i>Agropyron elongatum</i> <sup>28*</sup>			21		
<i>Agropyron elongatum</i> <sup>35*</sup>			10-12		
<i>Avena</i> <sup>7</sup>					
<i>Avena</i> <sup>21</sup>					

\* Obtained by subtracting 21, vulgare number, from hybrid number.

† Exclusive of *Ae. cylindrica*.

by subtracting the number of single chromosomes represented by the pairs from the sum of the parental numbers. Shaded part of circle within rectangle represents proportion of ring pairs (based usually on 'authors' statement, in few cases on illustrations only). Shaded part of small square within rectangle represents fertility (unshaded square representing less than 1 per cent fertility). Varietal and specific crosses are inside heavy-lined rectangles, generic crosses outside.

Ae. 7	Ae. 14	Ae.cyl 14	Ae. 21	Aeglt. 28	Sec. 7	Secltr 28	Avena 14	Avena 21	0
		6 □ ○							0 □ ○
		0-3 □ ○							
		7 □ ●							0 □ ○
				11,12 □ ●					
4-7 □ ●		6,7 □ ○							
6,7 □ ○	3-11 □ ●	5-11 □ ●							
	6-9 □ ○								
	13 ●			28 □ ●					0 □ ○
	4-7 □ ○	4-7 □ ○			7 □				
						28 □ ●			
					7 □ ●				
							7 □ ●	4-7 □ ○	
							5-10 □ ○	21 □ ●	

spasmodic and apparently readily influenced by external conditions. The much discussed *Triticum* ( $n=21$ )  $\times$  *Aegilops cylindrica* ( $n=14$ ) cross is a rather outstanding exception in that a mode of 7, mostly ring, pairs are observed at reduction division, suggesting strongly that a genom of 7 chromosomes (set C) in *Ae. cylindrica* is homologous with a genom of 7 in vulgare wheat, and that hence these two plants have one 7-chromosome ancestor in common (diagram I). This theory of relationship of *Aegilops* to vulgare wheat has met with considerable criticism, based usually on equally theoretical foundations. However, Senjaninova-Korczagina (105) thinks that the chromosome morphology of *Aegilops* does not speak in favor of its acceptance.

Recently, species of the genus *Agropyron* (or wheat grass) have been considered amenable to hybridization with wheat. The agropyrons are wild grasses, a few sometimes troublesome weeds and most of them serving useful grazing or forage purposes. The chromosome number is known for approximately one-third of the 60 species (7). According to Peto (92), *Agropyron* has attained a polyploidy above that of the "small grain" cereals. Of the cytologically known 7-,<sup>2</sup> 14- and 21-chromosome species, the majority are in the 14-chromosome group. *A. cristatum* has strains with 7 and 14 chromosomes, respectively; *A. smithii* has strains with 14 and 28, and *A. elongatum* has 35 chromosomes. The report of Sapéhin (100) indicates that a strain of *A. elongatum* possesses 28 chromosomes. Avdulov (2), in his study of chromosome morphology and systematics of grasses, reports finding several types of chromosome sets.

The cytology of interspecific crosses in *Agropyron* is unknown. Cytological study of intergeneric hybrids with *Agropyron* has barely begun. Sapéhin (100) has recently published results of four of these intergeneric crosses. The results are at variance, as table I shows, and Sapéhin explains the discrepancy on the basis of the employment of different biotypes in the two species of *Agropyron*, and offers the comment that the biotypes require more serious botanical and cytological analysis. This initial work will probably open up an interesting and profitable field of investigation.

*Haynaldia*, according to Bews (7), comprises two species, *H.*

<sup>2</sup> To simplify presentation the reduced number (in some cases derived from somatic number of original reports) is given.

*villosa* Schur. from the Mediterranean region and *H. hordeacea* Hack. from Algiers. *Haynaldia villosa* ( $n=7$ ), under the name of *Triticum villosum*, appears in crosses with 14-chromosome *Aegilops* (8) and in this rôle does not cytologically distinguish itself from a 7-chromosome *Triticum*. However, its difficulty in crossing with 7-chromosome *Triticum*, as shown by Oehler's tables (88), and the complete sterility of the cross when acquired, as reported by Sando (98), as well as the almost complete failure in pairing when crossed with 14-chromosome wheat, as found by Berg (5), probably bespeak a more distant relationship to *Triticum*.

## RYE

*Secale* is a small genus with 2 to 6 species including sub-species (7, 104). The outstanding species are *S. cereale*, the bread ryes, and *S. montanum*, a perennial grass. No polyploidy is known in rye and the only deviation from the number 7 is the occurrence in the species of segregates with 8 chromosomes. Belling (3) and Gotoh (29) are of the opinion that the extra chromosome is a segment from one of the largest chromosomes of the normal 7. Lewitsky *et al.* (69) and Hasegawa (32) contend that the 7 chromosomes are the same in both 7- and 8-chromosome rye and that the 8-chromosome rye possesses an extra smaller chromosome with the spindle attachment near one end instead of near the middle. Whatever may be the origin of the smaller chromosome, the frequently unequal distribution of the members of the small pair at meiosis, or according to Hasegawa at the first division in the pollen grain, leads to development of individuals with 14, 15 and 16 somatic chromosomes. Gotoh and also Lewitsky obtained individuals with 18 somatic chromosomes.

Meiosis in the interspecific cross *S. cereale*  $\times$  *S. montanum* is regular (70). According to Lewitsky (66), the chromosome morphology of *S. montanum* is similar to that of *S. cereale*. One pair of chromosomes is distinguished by satellites, the other by size and comparative length of the two arms. Of the approximately 30 intergeneric crosses involving 12 different species combinations, the largest number is with *Triticum* (1, 8, 9, 21, 23-26, 43), 5 crosses are with *Aegilops* (4, 43) and one, recently reported by Favorsky (19), is with *Agropyron*. If the degree of pairing is a true test, *Secale* is more closely related to *Aegilops* and *Agropyron*

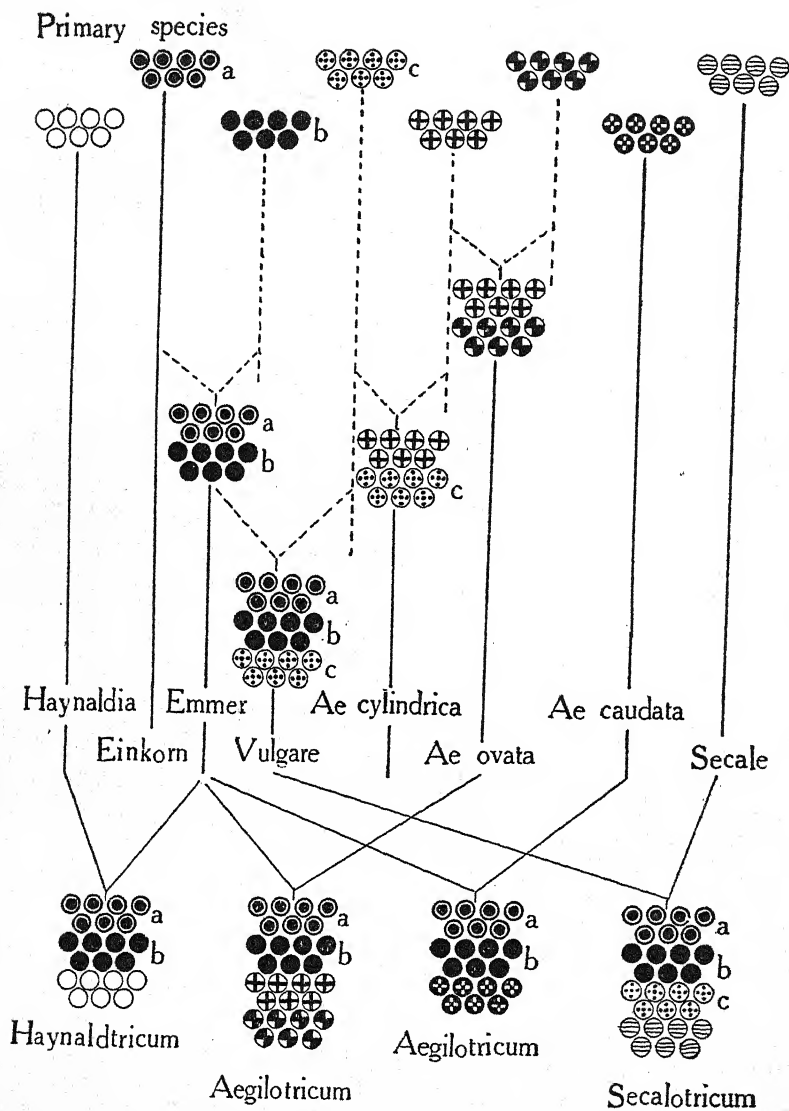


DIAGRAM 1. Allopolyploidy in wheat. Symbols indicate immediate origin of each set of 7 chromosomes. Thus in *Secalotricum* chromosome sets a, b, and c are identical with sets a, b, and c of its vulgare parent, while the remaining set is identical with that of its rye (*Secale*) parent. When back crosses have been made as in case of *Haynaldtricum* and *Aegilotricum* (table 1), the number of chromosome pairs has been found to approximate rather closely to the number of chromosomes in the hybrid that find identical partners in the parent with which it is back crossed. Unbroken lines represent the formation of amphidiploids, or new species (4 plants near bottom of diagram), through very recent artificial hybridization. Broken lines indicate hypothetical formation of species as found in nature, through possible natural hybridization. These hypotheses as to the origin of the natural species and suggested by pairing behavior in artificial crosses are not fully accepted by all research workers in the field.

than to wheat. Favorsky comments in respect to the *Triticum-Agropyron* cross that the 7 pairs, including ring forms, indicate a certain degree of relationship between rye and *Agropyron cristatum*.

#### BARLEY

The chromosome number has been ascertained for the majority of the approximately 25 species of *Hordeum*. Most of the wild species and all the cultivated varieties have 7 chromosomes. From man's viewpoint the genus *Hordeum*, in contrast to *Triticum*, has not been improved through polyploidy. Three species of *Hordeum* have 14 chromosomes, *H. murinum* and *H. jubatum* are both credited with 7 and with 14 chromosomes, and *H. nodosum* with 7 and with 21. Possibly, as in *Aegilops*, these species may comprise races or varieties which differ in chromosome numbers. The chromosome morphology is presented by Ghimpu (27), Lewitsky (66) and others. Two pairs of chromosomes are distinguished by satellites in varieties of the 7-chromosome barleys, *H. vulgare* and *H. distichum*, according to Lewitsky. The cytology of meiosis of *Hordeum* hybrids is not known.

#### OAT

Malzew (71), in his recent revision of the genus *Avena*, gives 7 species and 22 sub-species. The chromosome number is known for most of the species and many of the sub-species. Several species have 7 chromosomes. *A. strigosa* includes sub-species with 7 and with 14 chromosomes. The more important agricultural forms are included in the two species and 14 sub-species with 21 chromosomes. Increase in polyploidy has greatly increased polymorphism as to number of forms, bringing about a contrast to the extreme poverty of forms in the 7-chromosome species. Emme (18) finds considerable size variation in *Avena* chromosomes. Comparative arm lengths of chromosomes, more rarely appendages, assist in the comparison of genomes of different species.

Reports are published on 17 interspecific crosses of *Avena*, including 13 different species or sub-species (23-26, 17, 80, 84, 86, 94). The 21- $\times$ 21-chromosome crosses show comparatively few irregularities, in the form of unpaired chromosomes and trivalents, and have a fertility equal to that of the parents. Crosses involving



unequal numbers in parents are highly sterile. In contrast to *Triticum*, pairing in a  $14 \times 7$  cross is more regular, with more ring conjugants, than in  $21 \times 14$ . The  $21 \times 7$  cross gives fewer ring conjugants than the comparable combination in *Triticum*. The 21-chromosome *Avena* species, apparently closely related *inter se*, are more distantly related to the 14- and 7-chromosome species thus far used in hybrids. The 14-chromosome species are probably of more recent origin through the crossing of two 7-chromosome species. The 21-chromosome species, on the other hand, have originated from a more ancient  $7 \times 7$ -chromosome cross which later combined with a 7-chromosome species. Malzew (71) presents a diphyletic scheme giving a separate origin to the 21-chromosome section. The substitution of other 7- and 14-chromosome species in the above crosses, and also crosses within the 7-chromosome group as well as within the 14-chromosome group, may give added cytological information.

#### HYBRID OFFSPRING

The presence of univalents and other irregularities during maturation divisions gives rise to pollen grains and embryo-sacs of varying chromosome numbers. Even close hybridization tends, however slightly, to disturb chromosome balance in the offspring. In crosses involving different chromosome numbers in parents, and maximum pairing, as in the  $21 \times 14$ -chromosome wheat cross, the maturing  $F_2$  individuals lie too predominantly within one or the other of the parental chromosome groups, and the variants from the parental numbers are further eliminated in succeeding generations. Occasionally, stable lines are found with an intermediate chromosome number or with one chromosome more or less than a parental number. Sometimes the addition or loss of a chromosome manifests itself by some morphological character such as dwarfness (60). Some speltoids and compactoids of wheat and fatuoids of oats are brought out by the loss or gain of a particular chromosome.

Considerable study has been directed toward cytological aspects of dwarfs, speltoids, compactoids and fatuoids in general (31, 36, 37, 81, 85), and theories have been proposed as to the cause and origin of the various types of segregates.

Several factors seem to encourage reversal to parental chromo-

some numbers in hybrid offspring. Thompson and Armstrong (111) found in the  $21 \times 14$ -chromosome wheat cross that pollen grains with intermediate chromosome numbers are retarded in their nuclear development and this is again correlated with a deficiency of cytoplasm. "Unfavorable chromosome conditions in grains with intermediate numbers cause a complete abortion of some grains and retardation of nuclear development in others. Under the best available experimental conditions only 11 or 12% of  $F_1$  pollen grains germinate, in contrast to 70 or 80% for parental pollen. No grains with reduced cytoplasm germinate, and at least 50% of those with apparently normal cytoplasm fail to germinate." A large percentage of the apparently normal  $F_1$  pollen grains fail to germinate on the  $F_1$  stigmas, apparently the result of mutual unfavorable relationships of  $F_1$  pollen and  $F_1$  stigmas. Parental pollen germinates equally well on parental and  $F_1$  stigmas. Thompson (108), in order to determine the causes for wrinkled endosperm, back-crossed the  $F_1$  of  $21 \times 14$ -chromosome wheat crosses with emmer ( $n=14$ ) or vulgare ( $n=21$ ) pollen. By counting chromosomes in the offspring, he calculated the chromosome number in the nuclei in the functioning embryo-sac. He concluded that the endosperm condition is dependent on the extra 7 vulgare chromosomes in the triple fusion nucleus. It is plump when the extra 7 are absent or are in complete or almost complete duplicates or triplicates, *e.g.*,  $(14+14)\text{♀} + 14\text{♂}$ , or  $(21+21)\text{♀} + 14\text{♂}$ , or  $(21+21)\text{♀} + 21\text{♂}$ . It is shriveled when the extra 7 are haploid, *e.g.*,  $(14+14)\text{♀} + 21\text{♂}$  or are diploid or triploid for a few only. "The farther the chromosome situation departs from the complete absence or complete diploidy or triploidy of the vulgare chromosomes, the severer is the shrivelling. Endosperm conditions, depending in this way on chromosome conditions, play a large part in the non-appearance of many types in  $F_2$  and later generations of ordinary crosses." Wakakuwa (122), in a study of the  $F_1$  embryo development in wheat, concludes that embryos of all combinations between species with different chromosome numbers are smaller than those of the mother selfed. Growth of the embryos is always better if the female is the high-chromosome plant than in the reciprocal. Development of hybrid seed depends upon growth of both the embryo and endosperm, which depends, in turn, upon the numerical chromosome relationship of the sperm nucleus to the egg

and polar nuclei; respectively. Katayama (45), in a study of compatibility in reciprocal crosses of wheat and *Aegilops*, concludes that there is a qualitative as well as a quantitative chromosome relationship that determines development of the hybrid seed, and that the qualitative shows up more definitely the more remote the cross. Kihara and Wakakuwa (53, 61, 62), Nishiyama (84) and Watkins (123) offer more extensive discussions on fertile and sterile combinations relative to formation of hybrid offspring.

Some  $F_1$  hybrids, like wheat  $\times$  rye, are apparently completely pollen sterile. The anthers are poorly filled with irregular and a large percentage of abortive pollen grains and do not open. From these hybrids progeny may be obtained by back-crossing. In such cases, the chromosome number tends to stabilize when it acquires the chromosome number of the pollen parent.

In general, it seems that the fewer the conjugants in the meiosis of a hybrid the greater is the tendency for the offspring to attain, or even to far exceed, the chromosome number of the highest-chromosome parent, or, if the parents have the same number, to waver between the parental number and the sum of the parental numbers.

This brief presentation can merely suggest the general cytological trend in hybrid offspring. A longer list of literature is available than can be included (9, 21, 30, 76, 77, 95, 96, 112, 121, 125) and many articles represent more intensive study in the field of genetics.

#### AMPHIDIPLOIDS

The term amphidiploid has been applied to hybrid offspring arising through doubling of each genom of the  $F_1$  hybrid. All amphidiploids thus far known have developed from crosses showing few, if any, conjugants in the  $F_1$ . They are of interest in that they give us one illustration of how new species, especially allopolyploids, may be obtained. The new forms are comparatively fertile, in most cases quite stable, and combine characters of the two parents, a feat not readily accomplished between wide crosses. Pairing is not so regular as in either of the two parents. Whether this irregularity will gradually give way to regularity remains for time to determine.

The first two amphidiploids were described in 1926 by Tschermak and Bleier (117) as occurring in the  $F_2$  and  $F_3$  progeny of *Ae. ovata* ( $n=14$ )  $\times$  *T. dicoccoides* ( $n=14$ ) and *Ae. ovata*  $\times$  *T. durum*.

They were named *Aegilotriticum forma fertilis* No. 1 ( $n=28$ ), and *forma fertilis* No. 2 ( $n=28$ ), respectively. Percival (90) in 1930 discovered another octoploid in the  $F_2$  of *Ae. ovata*  $\times$  *T. turgidum*, and Kihara (54) in 1931 discovered still another in the  $F_2$  progeny of *T. dicoccoides*  $\times$  *Ae. ovata*, and in 1934 Oehler (87) reported 3 octoploid individuals found in the  $F_2$  of *Ae. triuncialis*  $\times$  *T. dicoccum*. He named the new form *Aegilotriticum triuncialis-dicoccum* ( $n=28$ ). Oehler, in the same contribution, described some hexaploid amphidiploid plants found in the  $F_2$  of the triploid cross, *Ae. caudata* ( $n=7$ )  $\times$  *T. dicoccum* ( $n=14$ ), and suggested for this constant form the name *Aegilotriticum caudatadicoccum* ( $n=21$ ). The first amphidiploid hexaploid was described by Tschermak in 1930 (116). It occurred in the  $F_2$  progeny of *T. turgidum* ( $n=14$ )  $\times$  *T. villosum* (7) and was named *T. turgidovillosum* ( $n=21$ ), but as it involves the much cast about species *villosum*, Tschermak suggests also the name "*Haynaldtricum*" *turgidovillosum*. Its cytology has been reported in detail by Berg (5, 6).

In wheat-rye progeny several amphidiploids have developed. The first was referred to by Meister in 1928 (74) as appearing in the  $F_2$  of *T. vulgare* ( $n=21$ )  $\times$  *S. cereale* and was described cytologically by Lewitsky and Benetzkaja (68) in 1931. This form has been referred to as "*Secalotriticum Saratoviense*." Lebedeff (65) in 1934 reported several amphidiploids occurring in the  $F_2$  of *vulgare* wheat  $\times$  rye following an attempted back-crossing with rye pollen. Lebedeff suggests apomixis as a possible method of origin.

Berg (5) crossed the amphidiploid *Aegilotriticum* ( $2n=(14+14)_2$ ) with the amphidiploid *T. turgidovillosum* ( $2n=(14+7)_2$ ) obtaining, thereby, a quadruple hybrid, involving 4 species, and 3 genera if *villosa* is considered as a species of *Hanaldia*.

Many suggestions have been proposed as to the method of origin of amphidiploids. It has been proved for the majority of the above cited amphidiploids that they have arisen not later than in the  $F_2$  generation, and they have probably had their origin in the ovule of  $F_1$ , or fertility would have led to their detection in  $F_1$ . The most frequently offered suggestion is the fertilization of an unreduced egg by an unreduced male gamete. The unreduced gametes developed probably as a result of failure of the first reduction division, the second resulting in the splitting of all the chromosomes, thus

forming 2 unreduced instead of 4 reduced cells. That such unreduced eggs are developed has been rather definitely demonstrated. Meiotic figures, indicating failure of the first reduction division, have been frequently observed. More direct evidence is probably offered by back-crossing or by pollinating  $F_1$  with known pollen and thus calculating the chromosome number of the  $F_1$  egg.

Katterman (49), in back-crossing the  $F_1$  of wheat-rye hybrids with vulgare pollen, ascertained by counts in 50  $F_2$  plants that the functioning egg in  $F_1$  contains from 11 chromosomes below to 3 above the somatic number of the  $F_1$  and that the percentage of unreduced functioning ♀ gametes is high. Kostoff (63) pollinated the  $F_1$  *T. (dicoccum* × *monococcum*) with *T. vulgare* pollen and obtained two plants with the somatic chromosome number 42, or  $(14 + 7) + 21$ . Müntzing (78) obtained a similar type of hexaploid, but with rye as the 7-chromosome parent in  $F_1$ . That unreduced female gametes are not infrequently produced seems evident. The question still remains unanswered as to the accomplishment of fertilization when the anthers of the hybrid so persistently fail to open. It has not been proved, however, that the anthers or parts of them never open. Katayama (47) in 1935 observed anthers discharging pollen grains in a haploid *Aegilotriticum*. Amphidiploids, in fact, arise comparatively infrequently, or at least sporadically. Katterman (50) proposes simultaneous fertilization of the unreduced ♀ gamete by ♂ cells from both parents. Kihara and Lilienfeld (58) observed nuclear migration in young pollen mother cells and raise the question as to the possible relation of nuclear migration to polyploidy in pollen grains, but are aware of the preclusion of such phenomena in the single spore mother cell of the cereal ovule. Nishiyama (84) suggests doubling of chromosomes after fertilization as the origin of several autotetraploid plants found in  $F_4$  segregates of a  $14 \times 7$ -chromosome *Avena* cross.

Aside from the usual type of chromosome segregates and amphidiploids in hybrid offspring, there occasionally occur other fertile forms. Some of these are characterized by deletion of apparently entire genomes. Kostoff (64) found in  $F_1$  of *T. vulgare* ( $n=21$ ) × *T. monococcum* ( $n=7$ ) a fertile plant with 35 somatic chromosomes and assumed that the *vulgare* was pollinated with an unreduced *monococcum* ( $n=7$ ) sperm. At meiosis, the 14 *monococcum* chromosomes formed 7 pairs and the *vulgare*, as interpreted

by Kostoff, pairing *inter se*, formed 5–10 more pairs. The subsequent generation had 28 chromosomes through elimination of 7 extra *vulgare* chromosomes. Nishiyama (83) discovered a diploid ( $n=7$ ) in the progeny of a 42-somatic-chromosome ( $17_{11}+8_1$ ) plant derived from *Avena fatua* ( $n=21$ )  $\times$  *A. barbata* ( $n=14$ ) cross. The autodiploid was in part like the tetraploid parent. It crossed readily with 7-chromosome *Avena* species. Dusseau (15) observed in the  $F_2$  of an intervarietal *vulgare* ( $n=21$ ) wheat cross a plant with characters of durum ( $n=14$ ) but with the chromosome number of *T. monococcum* ( $n=7$ ). He proposes the name *T. haplodurum* and suggests that the plant confirms the existence of a common gene in *T. vulgare* and *T. durum*.

#### HAPLOIDS

Until recent years, haploids or parthenogenic plants were unknown in angiosperms. Due to irregular distribution of unpaired chromosomes at meiosis and resulting sterility, haploids are of very temporary existence, unless they are capable of vegetative propagation.

The first haploid in cereals was described by Gaines and Aase (22) in 1926. This plant arose following pollination of *T. compactum* ( $n=21$ ) with *Ae. cylindrica* ( $n=14$ ) pollen and, no doubt, resulted from parthenogenetic development of the *T. compactum* egg cell. Pairing at meiosis was practically absent, the univalents segregating by chance to the two poles. Indications of non-reduction were noted. The plant was normal *T. compactum* except that it was highly sterile. The few seeds gave rise to normal  $2n$  plants. Yamasaki (124), in strains derived from varietal crosses of *T. vulgare* ( $n=21$ ), found 3 plants, smaller in size and highly sterile. Root-tip counts proved them to be haploid.

Chizaki (11) in 1934 found a haploid plant in offspring from seed produced by retarded spikes of *T. monococcum* ( $n=7$ ) in a field in bloom at high temperature ( $32^{\circ}$ – $34^{\circ}$  C.). Chizaki proposes the following causes of its origin: (1) “. . . the high temperature acts as a physical stimulus on the egg cell to start in the parthenogenetic development; (2) the pollen or pollen tubes lose their fertilizing ability owing to the high temperature, but they stimulate the egg cell to develop to haploid plant; (3) by the influence of high temperature the egg cell was injured and the male nucleus

developed merogonously." Kihara and Katayama (46, 55) in 1932 discovered 3 haploids in *T. monococcum*. One arose voluntarily in a pure line of bagged plants, the other two as results of X-raying spikes at the reduction division stage. The authors suggest parthenogenetic development of the unfertilized egg. As in the 21-chromosome haploid, there was no pairing at meiosis. The plants were smaller than the diploid *monococcum*. One seed gave rise to a normal diploid plant. Katayama (46) found that haploid plants appeared in a field of ordinary culture to the extent of .48%, indicating that egg cells of *T. monococcum* can develop parthenogenetically. When spikes at stage of meiosis were X-rayed, the haploids increased to 5.26%; if pollinated, the number increased to 7.41%. If spikes with mature pollen were X-rayed and pollen from these spikes transferred to untreated pistils, emasculated 3 days before, the number of haploids rose to 17.58%. Untreated pollen transferred to X-rayed pistils gave no haploids, showing that germ cells cannot be activated to develop embryos by exposure to X-rays. Katayama states: "Generally, it is considered that some male nuclei derived from pollen or PMC which was X-rayed degenerate without fusing with egg nuclei, if they entered the ovule. But, by the stimulation of male nuclei, egg nuclei may have been activated to develop parthenogenetically. It may be conjectured also that the endosperms developed through either the similar process in the egg or normal triple fusion."

Namikawa and Kawakami (79) observed rather frequent occurrence of twin seedlings in  $F_3$  and  $F_4$  generations of *T. vulgare* varietal crosses, and learned further that the twins were not always identical as to chromosome number. In 19 grains both plants were normal autodiploid ( $2n=42$ ); in 8 grains, one plant was autodiploid and the other autotriploid ( $2n=63$ ); in one grain, one plant was autotriploid and the other autotetraploid ( $2n=84$ ); and finally, in one grain one plant was autodiploid and the other haploid, containing only 21 chromosomes in root-tip nuclei. Fertility of the autotetraploid was 80.5%, of the autotriploid 0.4-47.2%, and of the haploid 2%. The authors do not comment on the method of origin. The rise of autopolyploids suggests the possibility of a similar origin of amphidiploids. Johansen (40) found about 10% of haploid seedlings in a commercial lot of grains of *Hordeum*.

The antithesis of haploids and amphidiploids is well illus-



trated by the appearance of two haploids from seeds of bagged spikes of *Aegilotriticum* ( $n=28$ ), as described by Katayama (47). One plant was slightly hypohaploid in that it had lost the greater part of one chromosome. This deficiency seemed to express itself morphologically in a slightly reduced spike. Katayama describes the meiosis as similar to that of the original  $F_1$  (*T. dicoccoides*  $\times$  *Ae. ovata*), but the number of conjugants was smaller, and non-reduction in the form of restitution nuclei was more frequent, resulting in a larger proportion of plump pollen grains in the haploid, especially in the hypohaploid individual.

#### CHROMOSOME CONJUGATION

Table I gives a bird's-eye view of cereal crosses cytologically studied. It represents a very general summary of over 300 crosses, involving more than 150 different species combinations. An attempt has been made to record the most dominating mode. The original data represent observations of plants grown under frequently different ecological conditions in countries far removed from one another. The results are comparatively uniform, with most deviations apparently resulting from use of different varieties.

The value of chromosome conjugations at meiosis of hybrids as a measure of relationships has recently been discussed by Sax (103).

A few experimental data in cereals, illustrating how various factors may influence instability of conjugation, will be cited. Katayama (44), in a statistical study of a wheat-*Aegilops* cross, found that number of conjugants is not influenced by direction of the cross nor by selection of individuals. Diurnal and seasonal variations he attributes to temperature. In plants kept at lower temperatures, as 6° and 15° C., for twelve hours, meiosis was normal except for occasional massing of a few chromosomes; at about 25° C. the number of conjugants began to decrease; and at the highest temperature (38° C.) the division was greatly disturbed and bivalents could not be observed. Peto (93) induced chromosome aberration, in the form of fractures, by high temperature treatment of barley seed. This primary result will lead to meiotic aberration if fractures are permanent or if resulting fragments are transposed to other chromosomes as observed. Peto states that: "Importance is attached to the discovery that a large proportion of

the mutant cells are eliminated during growth of the plant. The principle that only the fittest survive seems equally true of cells as of individuals and groups of plants or animals."

Results of X-ray treatment manifest themselves in many types of aberrations. Translocations of chromosome parts and resultant altered chromosome morphology were observed by Lewitsky and Araration (67) in *S. cereale*. Katayama (48), in *T. monococcum*, conjectured that a small part from one chromosome grafted to the end of another led to formation of an open ring of 4 chromosomes. Manifestations in the plant were slenderness, lowered fertility (about 36%) and poor germination. In polyploid wheats, where vital genes may be duplicated in several genomes, the effect on the morphology and physiology of the plant may not be apparent. According to Stadler (107), ". . . seedling mutations are common in *Triticum monococcum* with 7 pairs of chromosomes, much less common in *T. durum* with 14 pairs, and rare in *T. vulgare* with 21 pairs." From Tascher's data quoted by Stadler, "irradiation induces partial sterility with high frequency in *T. monococcum* but the frequency is much lower in *T. durum*, and extremely low in *T. vulgare*."

While mutations may not find as ready expression in polyploids, the chromosome homology and pairing will probably be disturbed equally as much as in the monoplloid. Sapéhin (99), in X-rayed *T. spelta*, noted, besides various chromosome aberrations, the absence of conjugants.

Pairing irregularity increases with increased polyploidy, as illustrated in wheat. Amphidiploids are characterized by considerable irregularity at meiosis although each chromosome apparently has an homologous mate. We do not know the exact cause for these irregularities and we do not know that they will completely disappear with time.

There are also some evidences in cereals of genic control of conjugation. Ekstrand (16), in a two-rowed barley, *Hordeum distichum* ( $n=7$ ), found some sterile or partially fertile plants showing asynapsis of some of the 14 chromosomes. Huskins and Hearne (37) found that absence of the *C* chromosome pair of fatuoid oat dwarfs was accompanied by failure of chromosome conjugation and consequent sterility. He thinks that the *C* chromosome carries the gene for pairing. The factor is so nearly domi-

nant that absence of only one of the *C* chromosomes, as in the 41-chromosome (somatic) plant, results in only lowered chiasma frequency. Asyn-desis was found also in the 40-chromosome (somatic) dwarf speltoid wheats. Nishiyama (81, 85) found in *Avena fatuoids* ". . . that the long arm of the *C* chromosome ( $s_1$ ) contributes to normal pairing and its short arm ( $s_2$ ) to the cultivated characters of the grain."

### EXPLANATORY NOTES

Every vegetative cell of a plant possesses chromatic material in its nucleus which is exhibited in a certain constant number of chromosomes at cell division. Among cereals this number ( $2n$ ) is usually some multiple of 7. When sex cells are formed at meiosis (reduction division) they receive only half this number ( $n$ ). At fertilization the number is again doubled. According as a plant contains  $n$ ,  $2n$ ,  $3n$ ,  $8n$ , etc., number of chromosomes in its body cells it is known as a haploid, diploid, triploid, octoploid, etc., respectively. Plants with higher multiples are known as polyploids. When these various classes form sex cells (gametes) the chromosomes appear, at a certain stage of the reduction division, singly (univalents), in pairs (bivalents); in triplets (trivalents), etc. The chromosomes which are thus associated in groups are homologous to one another, *i.e.*, each is derived from a different parent but all influence the same characters. The influence of one or more of each group may be dominant over the others (recessive) with respect to some feature.

As they are borne on the plants, the grains or fruits of cereals are surrounded by small scale-like leaves. In so-called covered grains these surrounding bracts are not removed by threshing; in naked grains they are.

allopolyploid: a polyploid whose chromosomes do not usually form multivalents at meiosis but pair as far as their homologies allow them (Darlington).

a polyploid possessing unidentical sets of chromosomes derived from two or more plants of dissimilar origin.

Allopolyploids contain a complement of chromosomes derived from two or more plants of dissimilar origin, and it is upon the degree of similarity between these two or more kinds that the type of pairing and disjunction of the chromosomes and

hence the segregation of factors depend. (Sansome and Philp).

amphidiploids: plants showing the exact doubling of each chromosome of the  $F_1$  hybrid from which they have been derived, *i.e.*, plants possessing the sum of the somatic numbers of both parents of the hybrid.

apomixis: development of an individual from an unfertilized egg without sexual fusion, whether the egg be normally haploid, or abnormally diploid through failure of reduction division.

asynapsis: failure of homologous chromosomes to occur in pairs during reduction division.

asyn-desis: asynapsis.

autodiploid: a plant possessing two identical sets of chromosomes.

autotetraploid: a plant possessing four identical sets of chromosomes.

back-cross: cross-pollination between a hybrid and one of its parents.

biotypes: distinct physiological races or strains within morphological species.

chiasma (ta): fusion of homologous chromosomes at one or more points.

compactoids: mutants occurring in cultures of common wheat, *Triticum vulgare*, and possessing characters of *Triticum compactum*, club wheat.

conjugants: paired or otherwise joined chromosomes.

endosperm: the food-storage portion of a seed which nourishes the embryo. Its tissue is triploid ( $3n$ ), resulting from fusion of one male with two female nuclei.

fatuoids: mutants occurring in cultures of cultivated oats and possessing the characters of *Avena fatua*, wild oats.

fiber-attachment: spindle-attachment, the point on a chromosome joining the "spindle-fiber" and proceeding first to the pole during anaphase.

genom: the entire number of chromosomes inherited as a unit from a parent.

hypohaploid: plant with slightly less than haploid chromosome number.

merogony: development of an embryo from a portion of an egg without the functioning of the egg nucleus; sperm nucleus only develops.

non-reduction: reception by sex cells of the vegetative number ( $2n$ ) of chromosomes rather than half the number ( $1n$ ).

PMC: pollen-mother-cell.

restitution nucleus: a single nucleus formed with an unreduced number of chromosomes in place of two nuclei with reduced chromosome numbers where reduction division should occur.

satellite: a segment of a chromosome, terminal or intercalary, separated from the rest by one or two very narrow constrictions.

speltoids: mutants occurring in cultures of common wheat, *Triticum vulgare*, and possessing characters of *Triticum spelta*, spelt wheat.

translocation: transfer of a segment of a chromosome to another part of the same chromosome or to a different chromosome.

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# THE RELATION OF WEATHER TO FUNGOUS AND BACTERIAL DISEASES

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The subject of the correlation of weather to plant diseases has long been recognized as of considerable importance and, for some years past, research has gradually elucidated some of the factors involved in the causation of epidemics, many of which have serious economic consequences. "Weather" is rather vague but is useful as a collective term covering innumerable factors.

There seems little point here in entering into a long description of the many factors involved in the term "Weather" and the various ways in which these factors influence the disease complex. There are many excellent papers which survey this subject from various viewpoints (12, 13, 48, 89) and the writer in 1929 gave a brief summary of the more important work which had been carried out up to then (33). Since then Wilson (124) published a rather comprehensive bibliography, with a subject index, which has fulfilled a very useful function.

## GENERAL

Investigations on this subject prior to 1920 had not probed fundamental issues and in many cases much labor was expended in an attempt to correlate the whole weather complex with disease incidence without reference to the separate factors. However, it was soon realized that the weather-disease complex could not be understood until the separate meteorological factors comprising "Weather" had been fully analyzed and their influence on disease studied one by one. The first outstanding and well organized research on each meteorological factor in relation to disease was started at Wisconsin, using apparatus by which the factors could be automatically controlled (54, 56 and lit. cit. in 33). Detailed knowledge of the way fungi and bacteria were influenced in the attack on their specific hosts was rapidly obtained both at Wisconsin and at other centers, while parallel work continued on the influence of the same factors on the growth of the organisms concerned, a work which had been carried on for years without realizing its true significance in the etiology of disease. Jones and his

colleagues investigated also the conditions of growth of the host plants and found that where the range of a factor was common to both host and disease organism, there the disease could be expected in most cases (55). This phase of etiological research was mirrored in the new presentation of these facts in the textbooks which, previously, had been more concerned with taxonomy and symptomatology. Views have changed very much and it is being recognized better that no true knowledge of a disease problem can be obtained without taking into consideration every etiological factor. Etiology has been considered too narrowly as concerning the immediate and apparent cause, the parasitic agent, instead of the vast field really involved. Even though such factors as temperature, moisture, light and wind are recognized as influencing disease incidence and progress, it seems that these same factors in a year preceding the season concerned, the total past history of the plant, its genetical and physiological constitution, and other factors are much less readily recognized as playing a truly significant part in the final causation of disease. Various writers have protested against the narrow view of etiology and a better conception of this subject is to be found in Link's (59) recent paper. He rightly draws attention to the need for investigating the physiology, culture and genetics of a plant in health jointly with that of the diseased state. External and internal factors may cause disease directly without any interference of a living disease agent, such as insect or fungus; it will be necessary for the investigator of a fungous or bacterial disease to keep this subject in mind. What the writer would prefer to call the "ecological" factor in the etiology of a disease is often ignored, though quite well known. This "ecological" factor would define the relationship between the natural habitat of a plant, *i.e.*, xerophyte, hydrophyte, etc., and that in which the plant was found with a disease, an especially important point when the plant is grown for economic purposes. Jarvis (48) has also suggested that a study of ecological conditions as related to crop production would yield a better understanding of the incidence and control of plant diseases.

#### EPIDEMIOLOGY

One of the most important points when investigating epidemics of plant diseases is that of the measurement of disease. As pointed



out by Moore and others (33, 61, 71), any human estimation of disease intensity is very unreliable when there is no standardization of degrees of infection. Even two individuals who have worked in conjunction on the same disease will each arrive at a different estimate of the percentage of disease. For apple scab (*Venturia inaequalis*), Moore, in England (71), has worked out a "category" method of estimating damage, which has the advantage of being formed on a statistical basis, comparisons between affected trees being reasonably sound. In America, charts for various rusts have been in use for some time, showing the standard degrees of infection (10). An interesting point of much importance is the difference between "extensity" and "intensity" of disease; "intensity" has in the past incorrectly covered both meanings. In only a few cases may measurement of "intensity," *i.e.*, severity in unit infection, represent also "extensity," *i.e.*, distribution of infection (5). In the measurement of epidemics this difference will have to be more considered than in the past.

#### ANTECEDENT FACTORS

There are many factors which influence disease epidemics considerably in advance of the event, only a limited number of which can be mentioned:

1. Weather generally determines to a very large extent the production of fungus spores in the summer season and it is on this inoculum that the appearance of disease in the following season depends. As might be expected, almost every disease has been noted in the literature on this subject, recent references to this point being 2, 7, 19, 70 and 122 among many others.

2. Given the inoculum, the conditions which determine its survival are probably the key to the subsequent appearance of disease, an epidemic of which is impossible without viable inoculum, in spite of any favorable circumstances in the current season. Although moisture has a considerable influence on the survival of fungus spores and bacteria, it is generally subservient to the influence of temperature. The literature on this subject, both prior to 1929 and subsequently, is voluminous and very interesting.

Vine mildew (*Plasmopara viticola*) commonly appears epidemically after wet winters (14, 15) but perhaps the warmer temperatures concomitant with the rains have an additional effect on the

abundant survival and germination of the fungus. A number of other Peronosporaceae are stated to be able to survive very low temperatures by means of their conidia, e.g.,  $-12^{\circ}$  C. for *Peronospora schachtii* (57). It seems, nevertheless, that in nature these conidia would not readily survive because the alternating low and warm temperatures, and dry and wet conditions, would sap their vitality much more severely than continuous low temperature. Most of these downy mildews usually survive by their oospores. Interesting work on the longevity of conidia of *Peronospora nicotianae*, *P. parasitica* and *Bremia lactucae* in Australia (1) demonstrated viability under dry conditions at low temperatures, especially if humidity was not too low. Conidia of the first fungus survived two months in cool, dry soil. While Australian conditions may allow overwintering by conidia, it may not be possible in other countries (126). Generally speaking, the rusts can withstand low temperatures (17, 19, 39, 70, 83, 92, 97).

Cereal rust epidemics in India provided an interesting problem and it has been demonstrated that temperatures influence the survival of the spores very thoroughly. *Puccinia glumarum* cannot withstand the high temperatures of the plains but survives the summer at high altitudes of and above 7,000 feet. On the other hand, *P. graminis* and *P. triticina* cannot survive the winter temperatures at these cold, high altitudes (65, 66). In the same way *Erysiphe graminis* (64) cannot survive the high temperatures of the plains and the annual re-occurrence depends on the survival of the conidia on self-sown plants at high altitudes. In America, Stewart's disease of corn (*Aplanobacter stewartii*) is severe only after warm winter months where the three months (December to February) mean temperature summation is over 100. This allowed the survival of the bacteria in sufficiently viable and distributed numbers to start an epidemic (99, 129).

An interesting fact relating to the survival of rust spores is the effect of ultra-violet light. It has been found that germination suffers if uredospores of *Puccinia graminis tritici* are exposed to direct sunlight or to ultra-violet rays and the rays from the red end of the spectrum. But this happened only when white and orange colored spores were used; those with red or greyish-brown walls do not suffer to such an extent and it is supposed that the red pigment when present protects the cytoplasm from the injurious light-

waves (28, 29). Ultra-violet light is also lethal to the mycelium of *Erysiphe graminis* (44).

3. The wind distribution of spores and bacteria has been known and accepted for most diseases but in some cases its importance has been over-estimated. With various bacteria and especially with *Venturia inaequalis* and *V. pirina*, wind seems to be negligible as a distributing agent until rain has detached the spores. There is no doubt, of course, that wind soon carries spores considerable distances but with the above fungi it was not sufficient to disturb the spores on the host tissues (107, 123). Wind velocities, artificially produced, of 300 to 500 m. per second were needed to detach scab conidia but only 60 m. per second were needed for *Cladosporium fulvum* and *Monilia fructigena* (121), while dropping water effected detachment very readily. This is a confirmation of the wind-blown rain experiments of Faulwetter in 1917 (see 33). Of course, rain does not only detach spores but also is a major factor in the expulsion of ascospores and pycnospores and is especially related to the presence of spores of *Venturia inaequalis* in orchards, the first appearance of which is the first clue for those responsible for the spray-warning service to be mentioned later (46, 110). (See also 33 for further details.)

The distribution of spores by wind, the use of aeroplanes in this study, and the various means for determining the presence of spores in the air, especially in crops and in orchards in relation to time of spraying, have been reviewed before (33). But it is of interest that spores have been recorded since even up to nearly 20,000 feet (69, 82) and that the air over the ocean is apparently free of spores (9), while the colder Arctic regions have very few spores (67, 68). In Russia, following the example of the United States and Canada (91), the aeroplane is being used to a considerable extent to examine the air for cereal rust spores (94). The aeroplane is also used in Great Britain and other countries for examining the fungal flora of the air. Cereals being tested for resistance to a rust form may be classed as resistant if no disease appears thereon. Whereas the case may really be that there are no spores of the rust in question in the air. Aeroplane exploration of the air to determine this point helps to form correct conclusions regarding resistance.

The rate of fall of spores in relation to wind distribution has

received attention for some time and calculations were made by McCubbin and Weston (see 33). Recently, Ukkelberg (111), using better apparatus, has tested the rate of fall of four cereal rusts and calculated their dispersal in a 30 mile per hour wind on the assumption of reaching 500 feet.

The epidemiology of cereal rusts is a subject on its own and cannot be adequately discussed in a few sentences. Only a few points have and can be raised herein but Stakman and Peltier have discussed this subject at length (79, 97).

#### DISEASE INITIATION AND EPIDEMICS

The initiation of disease and its development into an epidemic through the various stages of spore germination, infection, progress within the plant leading to collapse, production of secondary spores with their dispersal through the crop and finally the production of the over-wintering stage, all separately and collectively are bound up with meteorological conditions. The analysis of the numerous ways in which each of the factors influences the disease complex has been outlined before (33). Data on these points have accumulated to an overwhelming extent and cannot all be presented here. From the mycological papers published in the last six years which deal in any way with this subject (well over 600) only a few points can be mentioned.

It is very noticeable that much recent work on the germination of rust spores mentions, among other details, that better germination follows a scheme of freezing for several days, thawing, then wetting, perhaps followed again by drying and wetting (11, 50, 51, 100, 102). The exact scheme varies with the rust concerned but the idea remains the same (115). Some in investigating the black stem rust merely use naturally over-wintered material (92). Profuse germination of rust spores demands actual water and there is some reduction in the percentage germination in moisture saturated air or 99% and a great reduction below this degree of humidity (36, 42). Microscopic examination reveals a thin film of water round the spores in saturated air or 99% humidity. Very high humidity is also needed for the germination of other fungus spores (86, 116). It has been noted before (33) that many fungi, especially the rusts and downy mildews, need actual water for the germination of their spores and that a moisture saturated air was not

enough. Those writers who stated that 100% humidity was sufficient for germination probably had not examined for the presence on the spores of a thin film of water deposited in the saturated conditions—it probably was present. It is already known that spores dry out very quickly when exposed, the nature of the substratum influencing this considerably (53), but it is claimed that the germination of conidia of *Cystopus candidus* does not occur unless their own water-content becomes reduced by 50%. This is dependent upon the drying of the host tissues according to weather conditions (76). The same kind of relation is claimed for the conidia of potato blight (77). The temperature of production also influences the degree of germination; for example, *Puccinia graminis* teleutospores germinated better if produced at 55–60° F. than if produced at 70–75° F. (51). It is to be noted that very high humidities or saturated air are needed for infection and progress of disease, and this is generally true of downy mildews, potato blight and many Fungi Imperfecti (24, 113, 116).

There are several outstanding epidemics which are worth mentioning. Early blight of celery (*Cercospora apii*) depends on a combination of warm weather with heavy dew at night, no rain and light winds and, with the unusually high temperatures and relative humidity of 1931–32, the United States promptly suffered from a severe epidemic (98, 120). Mehta's work (64, 65, 66) on the cereal rust problem and cereal mildew has already been noticed in discussing the survival of spores. The occurrence of *Puccinia glumarum* each year at the foot of the hills has been correlated with the wind dispersal of uredospores from the infected self-sown plants in the hills, the uredospores being caught in aeroplane spore traps. The powdery mildew of the grape (*Uncinula necator*) in India is often epidemic in December when humidity and temperature is high but, in the districts where the monsoon is worse, the very heavy rains may check the intensity, though not the extensity, of the epidemic by washing off the infection (112).

The vine mildew (*Plasmopara viticola*) still continues to be a serious disease and is epidemic when continuous rains and temperatures of not lower than 13° C. prevail. High humidity does not seem sufficient for spore germination, an actual film of water being needed (14, 16, 74, 78, 85).

Apple scab (*Venturia inaequalis*) has been the subject of nearly

as much phenological research as potato blight and most of the recent work has aimed at obtaining data which could be used in a spray-warning service. The chief factor, perhaps, in this disease is the effect of early spring weather on the expulsion and germination of the ascospores, the production and germination of the conidia and the state of the leaves and buds. Wet periods long enough to produce the presence of spores in the air and to allow them to germinate, coupled with warm temperatures, lead to the initiation of an epidemic. While some state yet that the wind distributes the spores, others state that rain is the chief means of detachment of conidia, wind merely furthering the distance to which they may proceed. The whole phenological scheme fits in with the problem of when to spray to protect the susceptible tissues, usually the young, sappy tips just produced after heavy rains (40, 53, 62, 63, 72, 73, 121). Moore surveyed the phenology of this disease up to 1931 (72) referring to work already noted (33).

Potato blight (*Phytophthora infestans*) has been the subject of phenological research for a longer period than almost any other disease. Since Melhus put the biology of this fungus on sure ground, most of the work has either been a case of retesting or extending the scope of his discoveries or of applying them to the wider field of epidemics and the methods of forecasting their outbreak. Much of the literature has been noted (33) and Crosier (21, 22) has brought the subject almost up to date (1933), while his own researches have helped to complete some of the gaps in the knowledge of the biology of potato blight. Of particular interest is the study by Van Everdingen, using Miss Lohnis's material, in establishing a table of four requirements the fulfilment of which allowed a reasonable forecast of an epidemic of blight. These four conditions were: (1) a night temperature below dew point, of at least four hours' duration; (2) a night temperature not below 10° C.; (3) a mean cloudiness not below 0.8 on the following day and (4) rainfall of 0.1 mm., at least, on the following day. The Dutch Phytopathological Service succeeded in using Van Everdingen's four conditions for forecasting the liability of blight occurring and a spray-warning service has been in operation there since 1928. In Great Britain both Wiltshire (125) and Beaumont (4) have attempted to apply the Dutch rules for forecasting blight. But the detailed examination of meteorological data and the actual

outbreaks of blight showed that for that country the Dutch rules did not quite apply. Beaumont found that a better guide, in combination with the three other conditions, was a reading of the relative humidity at 3 p. m. instead of the night temperature below dew point. He was then able to correlate, in Devon, the incidence of blight with the occurrence of the four conditions through several years. As yet, however, a spray-warning service has not been established for this disease in Great Britain.

It is suitable to refer here to some serious attempts to place the science of the phenology of disease on a statistical basis. Tehon (105, 106) constructed graphs in which several meteorological factors could be used in combination and they indicated some promise; the writer has summarized the chief point in this work (33). There might be considerable use in Tehon's methods for some diseases and there is room for further investigation. The weak point in its use as a forecasting method is that for many, and usually the most important, diseases, the data used is too restricted and does not take into consideration the general biological facts, e.g., to take two cases only, one, physiological strains of cereal rusts and their wind distribution, and two, the four conditions necessary for potato blight epidemics.

#### PHYSIOLOGICAL SPECIALIZATION

Before passing on to the question of spray-warning services, it is worth mentioning the relation of physiological specialization to this subject. It is well known that many rusts are very specific and also that specialization to a variety of a host is possible. *Puccinia graminis tritici* is not only limited to wheat but many of its strains are limited to certain varieties or sets of varieties. Reed (88) has reviewed this subject recently. Further, it has been found that infection by many of these rust strains is affected by weather. For example, a given wheat variety may be susceptible to a strain of *Puccinia graminis tritici* in summer but resistant in winter or it may be susceptible or resistant according to the stage the plant has attained, i.e., seedling, vegetative or mature stages. Generally speaking, the temperature factor is the most important in this respect, though light and moisture have some influence. This subject is particularly important in the study of weather and disease, especially when this is designed to breeding new varieties



resistant in a given climate. In America, it is linked up with the aerial surveys for the presence or absence of certain rust spores at special times of the year in relation to the initiation and spread of epidemics. As Stakman points out, the discovery of spores in the air, blown from a district many miles away, is not necessarily a danger, unless the spores happen to be of a form or strain which will infect the particular variety of cereal crop which is being grown in that particular area (8, 37, 38, 43, 49, 52, 70, 75, 80, 90, 93, 96, 117, 119, 122).

#### PRACTICAL APPLICATION

There arises from the preceding discussion of epidemiology the question as to whether this increasing fund of knowledge can be used to control these diseases. It is now known that, for some diseases at least, given prompt meteorological and biological data, it is possible to forecast with reasonable accuracy the occurrence of an epidemic, though not the intensity or duration. A few countries have for some years developed an organization for the distribution of forecasts in relation to disease epidemics with the object of warning growers when to begin direct measures, such as spraying, against fungus and insect pests. The downy mildew of vines has been the subject of these spray-warning services for a long period and both France and Italy (60) have an extensive organization for this purpose. In only one of the vine districts of France, the number of subscribers using this organization is well above a thousand, a fact which demonstrates the usefulness of the service (128). Warnings are also given for powdery mildew and black rot of the grape, for apple scab and for potato blight. Holland's potato blight spray-warning service started in 1928 (125). In Canada and the United States, a spray-warning service against apple scab has now been in use for several years (20, 30, 81). Crosby gives a very detailed description of this organization as effected in New York State (20) and other states have services along the same lines. Of course, in the case of the spray-warning services in the United States, apple scab is only one of several troubles for which spray advice is given. There is little doubt but that this practical application of the research that has been carried out for years, on the phenology of diseases, will be extended to other diseases where control measures are possible both practically and economically.

## FUNDAMENTAL RESEARCH

In most cases the exact manner in which the environmental factors influence the disease are not known but, for some, explanations have been obtained. One of the most interesting cases is that of seedling blight of wheat and maize (*Gibberella saubinetii*), which has been investigated by Dickson *et al.* (see 33). The fungus has a very wide temperature range and the disease in wheat is worse at high temperatures (16 to 18° C. according to variety), while in maize it is worse at low temperatures (8 to 16° C.). Wheat is naturally a cooler climate crop than maize and at low temperatures it was presumed that abundant carbohydrates allowed cell-wall thickening which thus conferred a measure of resistance. At high temperatures the low sugar and dextrin content prevented a rapid cellulose formation with consequent susceptibility. Maize, on the other hand, reverses this process, sugars being more abundant at high temperatures. Recently (27), maize seedlings which were produced resistant to seedling blight were found to contain some 20% more uronic acids (associated with polyglucuronides) than susceptible varieties at the lower temperatures. Similarly, the attack by *Fusarium herbarum* on wheat was correlated with the presence of xylan and other hemicelluloses; the higher the percentage of xylan, the more readily did the enzyme of the fungus destroy the cell-walls (35). Further, wheat attacked by *Puccinia glumarum* and *P. triticina* is usually more heavily infected at low temperatures and seems to be correlated with the increase of albumin at low temperatures (34). Ravaz (84) suggests that, by increasing the sap concentration of vines, resistance to mildew would be conferred. But in another publication (114) this contention is reversed, as sap concentrations were not less in the susceptible than in the resistant varieties, though it was higher in the more mature leaves on any individual plant.

Another case showing physiological phenomena is that of *Basisporium gallarum* on maize (87). Low temperatures in maize do not allow active translocation to start, as already shown in the case of seedling blight. It has been shown that this fungus enters the seed at low temperatures because, translocation from the embryo not having started, there is still a neutral reaction which is not toxic to the fungus. At higher temperatures, translocation commences at once with germination, whence the tissues become

acid and therefore toxic to the fungus, conferring an apparent resistance to the plant. There are some newer varieties which, being able to grow at low temperatures and therefore having an early translocation from the embryo, are resistant to the disease.

In fire-blight of pears, the development of the bacteria is correlated with high humidity. One of the points of infection and dissemination is the nectar—bees, etc., visiting here leave bacteria which rapidly increase therein and the nectar becomes a source of infection for the same plant and others. At high humidity, the bacteria increase rapidly in the nectar but fail to do so at low humidity. This is because at high humidity the sugar content is only 1 or 2%, whereas at low humidity it is as high as 55%; at the high sugar content the bacteria not only cannot develop but they also lose their virulence (108, 109).

An interesting point, perhaps indirectly associated with this subject, is the work on *Botrytis cinerea* which demonstrated that apparently new and permanent variations could be induced by the action of very high temperatures (3). Many other workers have induced variation in fungi by high temperatures, X-rays and other methods (3a). While in nature the same conditions could not exist, it nevertheless indicates a field of investigation as to the possibility of environment slowly changing the parasitism or saprophytism of a fungus. There are two small points worth mention. Some workers have recorded that the leaves of plants diseased at the roots were several degrees warmer than those of healthy plants, indicating decrease in water supply (31). Microclimate and other investigations in crops should take this kind of fact into consideration. Also, with the recent work which is fast developing on fungal antagonism in the soil, it will have to be made certain that conclusions on the influence of soil temperature, moisture and other factors should not rather have been based wholly or partly on this phenomenon.

The vast complexity of this subject, very generally reviewed above, indicates that more and more it will be imperative that the plant disease complex should not be investigated from the narrower view of the immediate cause. Teams, working in close combination and consisting of mycologist, pathologist, geneticist, physiologist, statistician, etc., will have better chances of elucidating the work which remains to be done than isolated mycologists.

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